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July 2009

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Ethyl Lauroyl Arginate HCl

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

TABLE OF CONTENTS

FULL PUBLIC REPORT

Ethyl Lauroyl Arginate HCl

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cintox Australia Pty Ltd (ABN: 63 122 874 613)
38-40 George Street
Parramatta NSW 2150

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Lauric arginate
LAE
Aminat (containing 10 - 25% notified chemical)

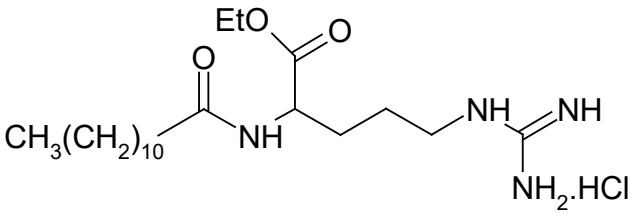
CAS NUMBER
60372-77-2

CHEMICAL NAME
L-Arginine, N2-(1-oxododecyl)-, ethyl ester, hydrochloride (1:1)

OTHER NAME(S)
Ethyl lauroyl arginate HCl (INCI name)
L-Arginine, N2-(1-oxododecyl)-, ethyl ester, monohydrochloride (9CI name)
Ethyl-N^{α}-dodecanoyl-L-arginate hydrochloride (IUPAC name)
N-α-lauroyl-L-arginine ethyl ester monohydrochloride
Monohydrochloride of L-arginine, N^{α}-lauroyl-ethylester

MOLECULAR FORMULA
$C_{20}H_{40}N_4O_3$. ClH

STRUCTURAL FORMULA



MOLECULAR WEIGHT
421.02 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, mass spectrometry, elemental analysis and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 85-95%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)

Chemical Name	Water		
CAS No.	7732-18-5	*Weight %*	<5
Chemical Name	Dodecanoic acid, ethyl ester (ethyl laurate)		
CAS No.	106-33-2	*Weight %*	<3
Chemical Name	Dodecanoic acid (lauric acid)		
CAS No.	143-07-7	*Weight %*	<5
Chemical Name	N^{α}-Lauroyl-L-arginine		
CAS No.	42492-22-8	*Weight %*	<3
Chemical Name	L-Arginine, ethyl ester, dihydrochloride		
CAS No.	36589-29-4	*Weight %*	<1
Chemical Name	L-Arginine, monohydrochloride		
CAS No.	1119-34-2	*Weight %*	<1
Chemical Name	Salts (mainly NaCl)		
CAS No.	-	*Weight %*	<2

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White powder

Property	Value	Data Source/Justification
Melting Point/Freezing Point	50.5 – 58.0°C	Measured
Boiling Point	Decomposes from 107°C at 101.3 kPa	Measured
Density	1110 kg/m^3 at 20°C	Measured
Vapour Pressure	5.45 x 10^{-7} kPa at 25°C	Measured
Water Solubility	> 247 g/L at 20°C	Measured
Hydrolysis as a Function of pH	Stable (half-life > 1 year) at pH 4,	Measured

	but susceptible to hydrolysis at pH 7 (half-life 57 days) and pH 9 (half-life 34 hours)	
Partition Coefficient (n-octanol/water)	log P_{ow} = 1.43 at 20°C	Measured
Surface Tension	25.43 mN/m at 19°C	Measured
Adsorption/Desorption	log K_{oc} = 1.76 at 20°C	Calculated
Dissociation Constant	The notified chemical is a hydrochloride salt that will be fully dissociated in the environmental pH range (4–9)	Literature data
Particle Size	Inhalable fraction (<100 μm): <8% Respirable fraction (<10 μm): 0%	Measured
Flash Point	>100°C	MSDS
Solid Flammability	Not highly flammable	Measured
Autoignition Temperature	Does not autoignite up to 400°C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Measured
Heavy Metal Analysis	Pb < 0.5 ppm Cd < 0.1 ppm Hg < 0.5 ppm As < 5 ppm	Measured

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity
Stable under normal conditions of storage.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported at < 25% concentration in propylene glycol or other solvents. It may also be imported in finished cosmetic and personal care products at concentrations up to 0.8%.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	5	5	5	5	5

PORT OF ENTRY
Sydney and Melbourne

IDENTITY OF RECIPIENTS
Cosmetic and personal care wholesalers, cosmetic salons, hair salons and retail outlets.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by ship in various pack sizes ranging from 5 kg plastic drums to 1000 kg totes. It will then be transported by road to the warehouses of cosmetic and personal care wholesalers for reformulation. The finished products will be packaged into various sized consumer packs (for example 300 mL to 1000 mL plastic bottles), packed in cardboard cartons, and then transported by road to end-users (cosmetic salons, hair salons, retail outlets).

When imported in finished products, the notified chemical is likely to be packaged in similar consumer packs and transported by road directly to end-users.

USE
The notified chemical is intended to be used as a preservative (up to 0.4%) and active ingredient (up to 0.8%) in cosmetic and personal care products. The product types in which it is intended to be an active ingredient include antimicrobial soap, anti-dandruff shampoo, deodorant, and oral hygiene products.

OPERATION DESCRIPTION
The notified chemical (< 25% concentration) will be manually weighed and transferred into a mixing vessel where it will be blended with other ingredients using automated mixing operations whilst the vessel is closed and sealed. The resulting blend (containing the notified chemical at concentrations up to 0.8%) will then undergo quality testing prior to being transferred by pump into a storage tank. The tank will be connected to a multiple head filler machine and the finished product containing the notified chemical automatically poured into plastic bottles, sealed and then packaged into cardboard cartons.

The finished products containing up to 0.8% of the notified chemical will be transported to distribution warehouses from which it will subsequently be supplied to retail outlets for consumer purchase, or to cosmetic salons, hair salons, etc.

The finished products containing the notified chemical will be used by consumers and professionals such as hairdressers or workers in beauty salons. Depending on the nature of the product these could be applied a number of ways such as by hand, using an applicator or sprayed.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport/warehouse	20	1	50
Cosmetic production	20	8	200
Professionals: cosmeticians, hairdressers etc	1000	8	200
Retail	1000	0.5	200

EXPOSURE DETAILS
Reformulation
Dermal, ocular and inhalation exposure of workers to the notified chemical (<25%) may occur during opening of the drums, weighing and adding the required amount of the notified chemical into a mixing vessel, and connecting and disconnecting transfer and filling lines. Dermal, ocular and inhalation exposure may also occur to concentrations of up to 0.8% of the notified chemical during quality control operations, and dispensing of the reformulated product into end use containers. Exposure is expected to be lowered by the enclosed nature of the mixing vessel, the automated systems used for mixing and dispensing, the use of local exhaust ventilation on the filling machines, and the wearing of personal protective equipment (PPE), including overalls, face-mask, safety glasses, safety shoes and impervious gloves. EASE modelling indicates very low levels of dermal exposure (without PPE) and potential inhalation exposure up to 0.025ppm assuming that local exhaust ventilation is utilised.

End-Use
Dermal, ocular, and inhalation exposure to the notified chemical (concentrations up to 0.8%) may occur in professions (e.g. hair dressers, workers in beauty salons) where the services provided involve the application of personal care products. Such professionals may use some personal protective equipment to minimise exposure, and good hygiene practices are expected to be in place. As such, exposure of such professionals is expected to be of either a similar or higher level than that experienced by consumers using products containing the notified chemical.

6.1.2. Public exposure
Members of the public may be exposed to the notified chemical in cosmetic products predominantly by the dermal route but also via inhalation, oral exposure (mainly by inadvertent ingestion of oral care products) and potentially by accidental ocular contact. Data on typical use patterns of a number of product categories in which the notified chemical is proposed to be used can be found in the Technical Guidance Document (TGD) on Risk Assessment of the European Chemicals Bureau (European Commission, 2003) and "The SCCP Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation" (SCCP, 2006). For the

purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe.

Dermal exposure
Dermal exposure is the main route of consumer exposure to the notified chemical from its presence in cosmetic and personal care products. Typical use patterns of selected cosmetics and personal care product categories that may contain the notified chemical have been used. Retention factors are also incorporated to allow for residual notified chemical remaining on the skin following use of rinse-off products (such as soaps or shampoos). Dermal exposure to the notified chemical was calculated as an internal dose which is proportional to use volumes, product retention factors, concentrations of the notified chemical expected to be present in each product type, and dermal bioavailability/absorption.

Default dermal absorption of 100% was assumed for calculation purposes (based on the default values outlined in the Technical Guidance Document on Risk Assessment of the European Chemicals Bureau (European Commission, 2003)). The actual level of dermal absorption may be lower than 100% based on the relatively high molecular weight of the notified chemical and that it is expected to be ionised at biologically relevant pH ranges. However, it also has surfactant properties and high water solubility, which may act to enhance its dermal absorption.

The internal dose resulting from dermal exposure to products containing the notified chemical was estimated using the below equation:

$$D_{int,derm} = \frac{A_{prod} \bullet n \bullet \frac{C}{100} \bullet \frac{B_{derm}}{100} \bullet RF \bullet CF}{BW}$$

Where:

$D_{int,derm}$	=	Internal dose via the dermal route, μg/kg bw/day
A_{prod}	=	Amount of cosmetic and personal care product applied to skin, mg/event
n	=	Frequency of product application, events/day
C	=	Concentration of notified chemical in product, %
B_{derm}	=	Bioavailability via the dermal route, %
RF	=	Retention factor
CF	=	Conversion factor, 1000 μg/mg
BW	=	Adult bodyweight, 60 kg

The calculated daily internal doses of the notified chemical from the use of different product types are shown in the table below.

Product type	**A_{prod}** (mg/event)	**n** (events/day)	**C** (%)	**RF**	**Daily exposure** (mg/day)	**$D_{int,derm}$** (μg/kg bw/day)
Leave on						
Deodorant	500	1	0.8	1	4	66.67
Body lotion	8000	1	0.4	1	32	533.33
Eye and face make up*	110	1- 2 (1.5 used for calcs)	0.4	1	0.48	8
Face cream	800	2	0.4	1	6.4	106.67
Foot spray	3000	2	0.4	1	24	400
General purpose cream	1200	2	0.4	1	9.6	160
Rinse off						
Bath products	17000	0.29	0.4	0.001	0.02	0.33
Facial masks	3700	0.1	0.4	0.1	0.15	2.47
Make up remover	2500	1	0.4	0.1	1	16.67

Shower gel	5000	1.07	0.4	0.01	0.21	3.57
Shampoo - antidandruff	8000	1	0.8	0.01	0.64	10.67
Soap bar – antibacterial**	800	6	0.8	0.01	0.38	6.4
Hair conditioner	14000	0.28	0.4	0.01	0.16	2.61
Hair styling products	5000	2	0.4	0.1	4	66.67
Shaving cream	2000	1	0.4	0.01	0.08	1.33
TOTAL						**1385.38**

* Sum of five different products: eye shadow; mascara; eyeliner; eyebrow pencil; and concealer
**It is assumed that consumers will use either the antibacterial/antiperspirant products or the regular products, rather than both, and as such, values for the regular products are not included separately.

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all product listed in the above table. This would result in a combined internal dose from dermal exposure of 1385 µg/kg bw/day.

Inhalation exposure
Inhalation exposure to the notified chemical from cosmetic and personal care products may occur via inhalation of spray aerosols such as anti-perspirant/deodorant sprays and hairsprays.

In order to estimate the internal dose from the use of these products, the following parameters were used in the calculations:

- Adult inhalation rate is 23 m^3/day (enHealth, 2003);
- Bioavailability via the inhalation route is 100%;
- The average body weight is 60 kg;
- Room volume of 2 m^3 to represent the volume of air immediately surrounding the user (European Commission, 2003); and
- Assumed exposure duration is 3.17 minutes - 10 seconds for actual spraying of the product and a further 3 minutes exposure after spraying (RIVM, 2006).

The equation used in the calculations of the internal dose via the inhalation route is shown below:

$$D_{int,inh} = \frac{A_{prod} \bullet n \bullet \frac{C}{100} \bullet \frac{B_{inh}}{100} \bullet t \bullet IR_{air} \bullet CF_1 \bullet CF_2}{BW \bullet V_{room}}$$

Where:

$D_{int,inh}$	=	Internal dose via the inhalation route, µg/kg bw/day
A_{prod}	=	Amount of deodorant or perfume spray, mg/event
n	=	Frequency of spray application, events/day
C	=	Concentration of notified chemical in product, %
B_{inh}	=	Bioavailability via the inhalation route, %
t	=	Time of contact (spray and exposure duration), minute
IR_{air}	=	Inhalation rate of person, m^3/day
CF_1	=	Conversion factor (time), 1 day/1440 minutes
CF	=	Conversion factor (amount), 1000 µg/mg
V	=	Room volume, m^3
BW	=	Adult bodyweight, kg bw

The typical use pattern and calculations of internal oral doses of the notified chemical for the deodorant spray and hair spray are shown in the below table.

Product Type	A_{prod} (mg/event)	n (events/day)	C (%)	Daily exposure (mg/day)	$D_{int,inh}$ (µg/kg bw/day)
Hair spray	10000	1-2 (1.5 used for calcs)	0.4	1.52	25.32
Anti-perspirant/ deodorant spray	3000	1-3 (2 used for calcs)	0.8	1.22	20.25
TOTAL					**45.57**

As a worst-case scenario estimation, if a person were exposed to both products listed in the table above, the combined internal dose from inhalation exposure is determined to be 45.6 µg/kg bw/day.

Oral exposure

Oral exposure to the notified chemical from cosmetic and personal care products may occur by inadvertent ingestion of products such as lipstick, toothpaste, mouthwash, etc. Typical use pattern and oral membrane exposure following use of such product categories have been used. Retention factors have also been incorporated, corresponding to the amount of residual retained on the lips or in the buccal cavity. Buccal absorption of 100% was assumed and a similar equation to that shown above for dermal exposure was used for the calculations. Estimates of child exposure from use of tooth pastes containing the notified chemical have also been included, using a higher retention factor (100%) and body weight of 10 kg (approximate average for 18 month old child) (RIVM 2006). The calculated daily internal doses of the notified chemical from the use of different product types are shown in the table below:

Product Type	A_{prod} (mg/event)	n (events/day)	C (%)	RF	Daily exposure (mg/day)	$D_{int,oral}$ (µg/kg bw/day)
Lipstick	10	4	0.4	1.0	0.16	2.67
Toothpaste	1400	2	0.8	0.17	3.8	63.4
Mouthwash	10000	3	0.8	0.1	24	400
TOTAL adult						**466.13**
Child toothpaste	1400	2	0.8	1.0	22.4	2240

If an adult were to use all product categories listed in the above table, a worst-case estimation of oral exposure to the notified chemical is calculated to be 466 µg/kg bw/day. Exposure to children via use of the notified chemical in tooth paste used by children is calculated to be 2240 µg/kg bw/day, which is expected to be an overestimate, given the high retention factor used in the calculations.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix B. Note that the values in this table have been adjusted to factor in the amount of notified chemical.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity (1) (90.1% notified chemical)	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute oral toxicity (2) (20.3% notified chemical)	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute dermal toxicity (90.1% notified chemical)	low dermal toxicity LD50 >2000 mg/kg bw
Rat, acute inhalation toxicity (0.63% notified chemical)	low inhalation toxicity at 0.63% concentration LC50 of formulation >5883 mg/m^3/4 hour*
Rabbit, skin irritation (90.1% notified chemical)	irritating
Rabbit, eye irritation (1) (99% notified chemical)	causes serious eye damage
Rabbit, eye irritation (2) (20.4% notified chemical)	severely irritating
Rabbit, eye irritation (3) (20.4% notified chemical)	severely irritating
Rabbit, eye irritation (4) (0.4% notified chemical)	slightly irritating
Rabbit, eye irritation (5) (0.04% notified chemical)	slightly irritating
Rabbit, eye irritation (6) (0.03% notified chemical)	slightly irritating

Rabbit, eye irritation (7) (0.02% notified chemical)	slightly irritating
Rabbit, eye irritation (8) (0.8% notified chemical)	slightly irritating
Guinea pig, skin sensitisation – adjuvant test (1) (20.4% notified chemical)	no evidence of sensitisation
Guinea pig, skin sensitisation – adjuvant test (2) (90.1% notified chemical)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days (1) (90.1% notified chemical).	NOAEL 3850 mg/kg bw/day male 4182 mg/kg bw/day female
Rat, repeat dose oral toxicity – 28 days (2) (20.3% notified chemical).	NOAEL 1070 mg/kg bw/day male 1187 mg/kg bw/day female
Rat, repeat dose oral toxicity – 13 weeks (3) (20.2% notified chemical).	NOAEL 671 mg/kg bw/day male 793 mg/kg bw/day female
Rat, repeat dose oral toxicity – 13 weeks (4) (89.4% notified chemical).	NOAEL 343 mg/kg bw/day male 398 mg/kg bw/day female
Rat, repeat dose oral toxicity – 52 weeks (5) (88.2% notified chemical).	NOAEL 271 mg/kg bw/day male 347 mg/kg bw/day female
Mutagenicity – bacterial reverse mutation (1) (89.4% notified chemical)	non mutagenic
Mutagenicity – bacterial reverse mutation (2) (20.3% notified chemical)	non mutagenic
Genotoxicity – in vitro mammalian cell mutation test (20.3% notified chemical) (1)	non genotoxic
Genotoxicity – in vitro mammalian cell mutation test (88.2% notified chemical) (2)	non genotoxic
Genotoxicity – in vitro metaphase chromosome analysis of human lymphocytes (20.3% notified chemical) (1)	non genotoxic
Genotoxicity – in vitro mammalian chromosome test in human lymphocytes (89.4% notified chemical) (2)	non genotoxic
Pharmacokinetic/Toxicokinetic studies (1): In vitro stability	Stable in simulated gastric fluids. Enzyme mediated hydrolysis to LAS and then to arginine in simulated intestinal fluids. Degraded to LAS (but not arginine) in human plasma and human hepatocytes.
Pharmacokinetic/Toxicokinetic studies (2): Metabolism in the rat	Rapidly metabolised by hydrolysis to arginine, then by natural amino acid catabolism is ultimately eliminated as carbon dioxide and urea in urine.
Pharmacokinetic/Toxicokinetic studies (3): In vivo and in vitro metabolism in the rat	Rapidly metabolised by hydrolysis to arginine, and then further catabolised to ornithine and urea.
Pharmacokinetic/Toxicokinetic studies (4): Pharmacokinetics in the rat	Rapid hydrolysis to LAS.
In vitro percutaneous absorption	Absorbed into pig skin
Preliminary determination of breakdown products in plasma after oral administration to healthy male volunteers	The notified chemical appeared to be relatively well-tolerated by the male volunteers. It appeared to degrade to LAS and arginine.
Determination of breakdown products in plasma after oral administration to healthy male volunteers	The notified chemical appeared to be relatively well-tolerated by the male volunteers. It appeared to degrade to LAS and arginine.
Toxicity to reproduction – Two generation study (88.2% notified chemical)	NOAEL 443 mg/kg bw/day female
Developmental toxicity study – rats (69.1% notified chemical)	NOEL dam 138 mg/kg bw/day NOEL foetus 1382 mg/kg bw/day
Developmental toxicity study – rabbits (69.1% notified chemical)	NOAEL dam 207 mg/kg bw/day NOEL foetus 691 mg/kg bw/day
Carcinogenicity	No data available

*Worst case value based on back calculation from amount of aerosol fraction collected in the breathing zone. Based on the sampling of the volatile fraction, the LC50 of the formulation was > 28150mg/m^3/4hr.

Toxicokinetics, Metabolism and Distribution

Dermal absorption of the notified chemical was measured in vitro using pig skin biopsies. Attempts were made

to measure the absorption of a formulation containing 0.4% notified chemical, which corresponds to the concentration at which it is proposed to be used in cosmetic products when present as a preservative ingredient. Under such conditions, the notified chemical could not be detected, as it was present at levels below the limit of quantification (ie. below 4.84 mg/L) in all analysed compartments. A more concentrated formulation (1.96%) was subsequently tested and the percutaneous absorption after an exposure time of 24 hours was found to be 5.24 ± 2.29 μg/cm^2. This corresponds to a total absorption of 5.52% into the epidermis and dermis. Systemic absorption is likely to be low.

There are no data available on the toxicokinetics or distribution of the notified chemical following inhalation exposure. The observation of clinical signs of toxicity, such as hypothermia suggests that absorption by the inhalation route may occur.

The metabolism, distribution, pharmacokinetcs and excretion of the notified chemical were examined in a number of in vitro and in vivo rat studies, and human studies.

In vitro rat studies
The notified chemical was incubated with S9 liver fractions and rat plasma. In the presence of S9, the major metabolite after 24 hours was found to be ornithine, though there were also quantities of arginine, Nα-lauroyl-L-arginine (LAS) and urea identified. Incubation of plasma indicated rapid hydrolysis of the notified chemical to LAS and arginine, then subsequently to ornithine.

In vivo rat studies
Following administration of a single oral dose of the notified chemical (radiolabelled) to rats, the distribution of the radioactivity of the dose was examined. Five days after dosing, a mean of 36.6% of the radioactivity of the dose was excreted as CO_2 in expired air, 11.8% in urine and 4.3% in faeces. A mean of 46.4% of the radioactivity of the dose remained in the carcass at sacrifice (3.4% in the liver, 2.0% in the gastrointestinal tract).

In a separate study plasma levels of the notified chemical and its metabolites were examined following single oral administration to rats. At all sample times, the notified chemical and LAS were present at levels of less than 10%. Arginine was found to be the major metabolite at shorter sample times. Extraction of radioactivity decreased over time. These results suggest that the notified chemical is rapidly metabolised in vivo to arginine and subsequently to ornithine and urea. The notified chemical and its metabolites are likely to bind to plasma proteins and/or are naturally incorporated.

The pharmacokinetics of the notified chemical was also examined in another study. Concentrations of the notified chemical were generally found to be low and variable, due to rapid hydrolysis to LAS, most likely in the gastrointestinal tract and by tissue and plasma esterases. As such, it is considered that the concentrations of LAS provide a better indication of relative systemic exposure and absorption of the notified chemical.

A toxicokinetic evaluation was also performed in conjunction with the 52 week oral repeat dose study. This revealed that exposure to the notified chemical did not increase in a linear fashion with increasing dose.

Human studies
In vitro experiments using the notified chemical were conducted using plasma, hepatocytes, and simulated gastric and intestinal fluids. It was found to be stable in simulated gastric fluids, whilst in simulated intestinal fluids it was relatively stable, though quickly degraded to LAS and arginine in the presence of pancreatin (indicating enzyme mediated hydrolysis). In human plasma and human hepatocytes, LAS was the only observed degradation product.

In addition, there were two studies that examined the breakdown products of the notified chemical in the plasma of male volunteers. Both studies indicated that the notified chemical degraded to LAS and arginine in humans.

Taken together, the toxicokinetic studies suggest that the notified chemical or its metabolites will be well absorbed from the gastrointestinal tract (Ruckman 2004). It will be rapidly metabolised in the body, initially by hydrolysis of the ester to LAS, followed by hydrolysis of the amide to arginine and lauric acid. Arginine will then undergo natural amino acid catabolism via the urea and citric acid cycles to form ornithine and urea and perhaps become incorporated into other endogenous products such as plasma proteins, then ultimately forms carbon dioxide. This suggests that the notified chemical is eventually eliminated from the body via urea in urine and carbon dioxide in expired air. This suggests that the notified chemical is metabolised in the body into

naturally occurring chemical species that can be degraded by normal mammalian biochemical pathways.

Acute Toxicity
The notified chemical was found to be of low acute oral toxicity in rats (LD50 >2000 mg/kg bw) based on two studies performed at different concentrations (20.3 and 90.1%). It was also of low acute dermal toxicity (LD50 >2000 mg/kg bw) according to a test performed with the notified chemical at a concentration of 90.1%.

The acute inhalation toxicity of the notified chemical was measured at concentrations of 0.63%. Under the conditions of the study, the notified chemical was found to be of low toxicity by the inhalation route at a concentration of 0.63% with no mortalities observed. However, the study authors indicate that, based on the "combined experimental evidence, the notified chemical may cause mild respiratory tract irritation if exposure to the aerosol is sufficiently high". In addition, based on the details described in the study, it is difficult to determine the actual concentration of the notified chemical that reaches the breathing zone. It is also noted that signs of respiratory distress were observed during the developmental toxicity studies (see below).

Effects associated with repeated inhalation of the notified chemical are not known.

Irritation and Sensitisation
The notified chemical was found to be irritating to the skin based on persistent desquamation observed in two animals. The neat notified chemical is also assumed to be severely irritating to the eyes, or perhaps corrosive, as severe irritancy effects were observed when it was tested at concentrations of 20.4%. When tested at concentrations similar to the levels at which it will be present in cosmetic products, slight irritancy effects were noted, but these were below the level for these mixtures to be classified as irritants.

In addition, there was no evidence of sensitisation in the two guinea pig maximisation tests performed on the notified chemical (20.4% and 90.1% test concentrations).

Mutagenicity
The mutagenicity of the notified chemical was investigated in several tests (though not an in vivo test). It was found to be negative when tested using the bacterial reverse mutation assay in two separate tests (at concentrations of 89.4% and 20.3%). However, it is noted that this assay may not be the most appropriate method for evaluation of the mutagenicity of the notified chemical, given its relatively high toxicity to bacterial cells. (EFSA 2007).

When tested using the in vitro gene mutation test in mouse lymphoma L5178Y cells in two separate studies (at concentrations of 20.3% and 88.2%) the notified chemical was found to be non-clastogenic. It was also tested in human lymphocytes cultured in vitro to determine its potential to cause chromosome aberration (in two separate tests at concentrations of 20.3% and 89.4%). It was found to be non-clastogenic, however, in the test at 89.4% concentration there was evidence of polyploidy-inducing activity, whilst the other test did not measure polyploidy. Given that these effects were only observed at cytotoxic doses their biological significance is questionable. This is further supported by testing performed on LAS, a metabolite of the notified chemical, in a mouse micronucleus assay, as described by EFSA (EFSA 2007). The study was performed by single oral gavage administration at 2000 mg/kg bw and sampling of the bone marrow after 24 and 48 hours. There was found to be no biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow. Therefore the notified chemical is considered to have low potential for aneuploidy induction.

Repeated Dose Toxicity (sub acute, sub chronic, chronic)
The repeat dose oral toxicity (administration via the diet) of the notified chemical was examined in two 28 day preliminary studies, as well as two 13 week studies and one 52 week study. There were a number of transient effects observed throughout each of these studies. The effects of particular note include the histopathological changes observed in the forestomach and alteration of peripheral haematological parameters, mainly concerned with decreases in white blood cell counts.

White blood cells effects were observed in both 13 week studies and the 52 week study. Generally, there was not a consistent pattern in the white blood cell types affected during these studies, the time scales in which they were observed, or consistent effects between rat strains and sexes, as well as there being little dose dependency relationships. In addition, there were no abnormal morphological changes in blood cells, treatment related effects on bone marrow, or histopathological findings associated with lymphoid tissue. For these reasons, the white blood cell effects may be considered to be of no toxicological significance. Alternatively, they may be related to

effects of the notified chemical on the forestomach. It is plausible that the reduction in the white cells in the peripheral blood may be the consequence of a physiological response to the stomach changes. However, it should also be noted that there is little correlation between the individual animals displaying the blood cells effects and those in whom the forestomach lesions were detected, although this lack of correlation may be due to the dynamic nature of the changes (Brown 2008). In summary, the white blood cell effects are of questionable toxicological significance.

Effects in the forestomach of the rats were observed in one of the 13 week studies and in the 52 week study. The findings were seen only in the forestomach region of the rats and were generally of low severity. The effects were dose related, though only of statistical significance at the highest dose levels. The effects were considered to be treatment related local irritation effects caused by administration of the notified chemical, perhaps as a direct effect on epithelial cells as a result of the surfactant properties of the notified chemical. Thus the occurrence of stomach lesions were not considered to be attributable to systemic toxicity. As the forestomach of rats does not have a protective mucus lining and there is no direct counterpart of the rat forestomach in humans, the forestomach findings may not be of relevance to humans. Nonetheless, the NOAEL associated with the stomach effects in the 52 week study (NOAEL 271 mg/kg bw/day male, 347 mg/kg bw/day female) was deemed appropriate to evaluate the risk of possible local effects from oral exposure.

Carcinogenicity
No testing available.

Developmental Effects
The notified chemical was tested for developmental effects in both rats and rabbits and showed no teratogenic effect in either species.

In the rat, the NOEL for the dam was established as 138 mg/kg bw/day of notified chemical, based on deaths at 415 and 1382 mg/kg bw/day. It is noted that these deaths may have been a result of an indirect effect of dosing of animals. The NOEL for the foetus was established as 1382 mg/kg bw/day of notified chemical, based on no adverse effects observed at the highest dose tested (1382 mg/kg bw/day).

In the rabbit, the NOEL for the dam was established as 69 mg/kg bw/day of notified chemical, based on signs of respiratory distress and deaths at 207 and 691 mg/kg bw/day. It is noted that the deaths and signs of respiratory distress may have been as a result of an indirect effect of dosing of animals.

Despite the slightly higher risk of irritation to the respiratory tract at doses of 207 mg/kg bw/day and above, it was concluded that 207 mg/kg bw/day of notified chemical was the NOAEL for the dam. Effects on body bodyweight gain and food consumption were also observed at 691 mg/kg bw/day. The NOEL and NOAEL for the foetus was established as 691mg/kg bw/day of notified chemical, based on no adverse effects observed at the highest dose tested (1382 mg/kg bw/day).

The respiratory distress observed in these studies is not likely to be a systemic response to oral ingestion of the notified chemical, though it may suggest bronchial irritation upon inhalation of the notified chemical.

Toxicity for Reproduction
The effects of the notified chemical on reproductive performance were examined in a preliminary one generation study, as well as a two generation study. A finding of particular note in both of the studies was the delay in vaginal opening observed at the highest dose level, indicative of delayed onset of puberty in female rats. Whilst this effect did not have any lasting impact on the normal sexual development of the animals, and the mechanism by which it occurs is unknown, it cannot be disregarded and is considered to be of potential toxicological significance. This suggests that a NOAEL value of 6000 ppm, corresponding to 502 mg/kg bw/day LAE (443 mg/kg bw/day notified chemical) is appropriate. In the absence of systemic effects in the chronic studies this NOAEL was deemed appropriate for risk assessment considerations.

Observations on Human Exposure
Pharmacokinetic studies were performed by single administration of the notified chemical to human volunteers at dose levels of 1.5, 2.5 and 5 mg/kg bw. The notified chemical appeared to be well-tolerated by the volunteers. The SCCP opinion on the notified chemical notes that the burning sensation in the throat and nausea experienced at the high dose level and the diarrhoea experienced at the low dose level may be due to mucosal irritation that may occur.

Health hazard classification
Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Xi; R38 Irritating to skin
Xi; R41 Risk of serious damage to eyes

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Exposure of workers to the notified chemical at concentrations of up to 25% may occur during reformulation processes (dermal, ocular, or inhalation).

Upon dermal contact with the notified chemical at concentrations up to 25%, skin irritation may occur. Upon ocular contact with the notified chemical, corrosion or serious eye damage may occur. Appropriate use of local exhaust ventilation and personal protective equipment, particularly safety glasses, overalls, face/dust mask, impervious gloves, and safety shoes during reformulation operations is expected to reduce exposure levels to the notified chemical and hence lower the incidence of such effects.

The acute dermal toxicity of the notified chemical was found to be low. Health effects resulting from repeated dermal exposure to the notified chemical have not been examined. However, on the basis of effects observed in test animals following repeated oral exposure, low toxicity following repeated dermal exposure is expected (see below discussion in public risk assessment section).

The effects of inhalation of the notified chemical at concentrations up to 25% have not been studied. However, based on testing at lower concentrations, it is possible that the notified chemical may cause respiratory tract irritation at higher concentrations. The effects associated with repeated inhalation exposure to the notified chemical are unknown. The use of local exhaust ventilation during handling processes and the low vapour pressure of the notified chemical is expected to minimise the levels of inhalation exposure experienced by workers.

Overall, the notified chemical is not considered to pose an unacceptable risk to cosmetic production and transport workers, given the use conditions described. However, employers should implement appropriate control measures to minimise dermal, ocular and inhalation exposure.

The risk for beauty care professionals who regularly use products containing the notified chemical (up to 0.8%) is expected to be of a similar or perhaps higher level than that experienced by members of the public who use such products on a regular basis, in light of the duration of exposure.

6.3.2. Public health
The public may come into contact with the notified chemical (< 0.8%) through the use of a range of cosmetic products via dermal, inhalation, oral or ocular exposure.

Local Effects – Dermal/Ocular
The notified chemical itself was found to be irritating to the skin. However, skin irritancy effects are not expected to occur at the relatively low concentrations at which the notified chemical will be present in cosmetic products used by consumers (< 0.8%). In addition, the notified chemical did not display skin sensitisation potential. Therefore, the notified chemical is not expected to pose a risk of skin irritancy or skin sensitisation as a result of use of cosmetic products containing it.

The notified chemical was found to cause severe irritation to the eyes. However, when tested at concentrations similar to that present in cosmetic products (up to 0.8%), slight irritation was observed. Therefore, the possibility of slight irritancy upon ocular contact cannot be ruled out. However, intentional ocular exposure is not expected, and rinsing of the eyes is likely in the event of accidental exposure. Overall, the risk to the public of eye irritation arising from use of cosmetic products containing the notified chemical is not expected.

Inhalation/Respiratory effects
Members of the public may be exposed to the notified chemical via inhalation as a result of the use of spray cosmetic products. The effects associated with repeated inhalation exposure to the notified chemical have not been investigated. The acute inhalation toxicity study performed on the notified chemical indicated mild

respiratory tract irritation, together with effects on breathing, such as laboured breathing patterns. In addition, the developmental toxicity studies indicated possible respiratory effects associated with inhalation of the notified chemical. Such effects may increase in severity upon repeated exposure, and other effects may emerge. As such, the effects associated with repeated inhalation of the notified chemical remain unknown and thus the risk is not considered to be acceptable.

Local Effects - Oral
The burning sensation experienced in the throat of two human volunteers who had been administered the notified chemical at doses of 5 mg/kg bw may be evidence of mucosal irritation in the oral cavity caused by the notified chemical. Similar effects were not experienced at the lower dose levels used in the human studies. In addition, the dose levels used in these studies far exceed the total oral exposure levels for adults that are expected to be experienced from use of oral care products and lip products. As such, effects of this type are not expected to be experienced by members of the public using these products.

Stomach irritancy effects were observed during one of the sub-chronic (13 week) repeat dose toxicity studies and in the chronic (52 week) repeat dose toxicity study, though not in the 28 day study. This indicates that stomach irritation may occur following repeated ingestion of products containing the notified chemical. Using the NOAEL of 271 mg/kg bw/day from the chronic toxicity study, the resulting margin of exposure (MOE) is 581, for adult oral exposure levels given in Section 6.1.2. For children using children's toothpaste products, the resulting MOE is 121. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. The MOE is based on conservative assumptions (e.g. 100% absorption, 100% retention factor for use of children's toothpaste) and likely overestimates the risk. In addition, there is some question as to whether the forestomach effects observed in rat repeat dose toxicity studies are necessarily of relevance for effects in humans, given that there is no human counterpart for the rodent forestomach.

Therefore, the risk to the public of local effects associated with the use of oral products containing the notified chemical is not considered to be unacceptable at this time.

Systemic Effects
Consumers, especially children, may accidentally ingest products containing the notified chemical, particularly oral hygiene products. Given the measured low acute oral toxicity of the notified chemical, acute toxic effects arising from accidental ingestion of the notified chemical are not expected to occur.

Combined oral, dermal and inhalation exposure to the notified chemical is estimated to be 1897 μg/kg bw/day. Based on the NOAEL of 443 mg/kg bw/day established in the two generation reproduction study, the resulting MOE is 234. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. The MOE is based on conservative assumptions i.e. that a person is exposed to all types of products containing the notified chemical and 100% bioavailability via all three exposure routes.

Therefore the risk of adverse systemic effects from use of products containing the notified chemical is not considered to be unacceptable.

Overall
At present the risk to the public from use of the notified chemical in dermal and oral products is not considered to be unacceptable. However, the notified chemical may be included in food in Australia in the future. Therefore additional exposure to the notified chemical from non-cosmetic sources may lead to exposure levels that may not be acceptable.

Considering the evidence of respiratory irritation following acute inhalation exposure to the notified chemical and the unknown nature of long term effects associated with repeated inhalation exposure, the risk of inhalation of spray cosmetic products containing the notified chemical is not considered to be acceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported in solution and reformulated into cosmetic and personal care products. Losses from reformulation (spills, equipment cleaning and container residues) may reach 3% of the imported quantity. This will be treated in on-site treatment plants, from which discharge to sewer is assumed based on the high water solubility.

RELEASE OF CHEMICAL FROM USE
Essentially complete release to sewer can be expected when cosmetic and personal care products containing the notified chemical are washed from the skin and hair.

RELEASE OF CHEMICAL FROM DISPOSAL
Only limited quantities (residues in import and consumer containers) will require disposal. The import containers are expected to be sent to drum recyclers where their residual contents will be destroyed by incineration. Residues in consumer containers, representing less than 2% of the imported quantity, may be disposed of to landfill with the containers, or washed to sewer when containers are rinsed by householders before introduction into the recycling stream.

7.1.2 Environmental fate

The notified chemical achieved a biodegradation plateau of about 45% after 5 days (not meeting criteria for ready biodegradability) in a closed bottle test, but was readily biodegradable in a modified Sturm test. Therefore, some biodegradation can be expected during sewage treatment, but residues may be discharged to receiving waters and disperse as the notified chemical is highly water soluble. There may be some sorption to sediment as the notified chemical is surface active. Sorption to organic matter is also likely as the notified chemical is an ammonium salt. The notified chemical is not expected to persist in receiving waters or to bioaccumulate in fish because of its biodegradability.

7.1.3 Predicted Environmental Concentration (PEC)

The PECs in receiving waters can be estimated as tabulated below based on the hypothetical worst-case assumption that all of the notified chemical will be discharged from sewage treatment plants. Actual exposure concentrations are expected to remain below these estimates as the notified chemical is biodegradable.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment	
Total Annual Import/Manufactured Volume	5000 kg/year
Proportion expected to be released to sewer	100%
Annual quantity of chemical released to sewer	5000 kg/year
Days per year where release occurs	356 days/year
Daily chemical release:	13.7 kg/day
Water use	200.0 L/person/day
Population of Australia (Millions)	21.374 million
Removal within STP	0%
Daily effluent production:	4,275 ML
Dilution Factor - River	1.0
Dilution Factor - Ocean	10.0
PEC - River:	3 µg/L
PEC - Ocean:	0.3 µg/L

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 hours)	EC50 = 23.7 mg/L	Harmful
Daphnia Toxicity (48 hours)	EC50 = 6.5 mg/L	Toxic
Algal Toxicity (72 hours)	E_bC50 = 0.46 mg/L	Very toxic
Inhibition of Bacterial Respiration	EC50 = 98.5 mg/L	

The notified chemical is harmful to fish, toxic to daphnids and very toxic to algae, based on these test results. The slight inhibitory effects in sewage sludge bacteria that were evident at concentrations of 30 mg/L and higher are of no practical significance, given the freshwater PEC of 0.003 mg/L calculated above.

7.2.1 Predicted No-Effect Concentration

The PNEC can be calculated by application of an assessment factor of 100 to the most sensitive test result as acute data are available for three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
Algal toxicity	0.46 mg/L
Assessment Factor	100
Mitigation Factor	1.00
PNEC:	4.6 µg/L

7.3. Environmental risk assessment

The risk quotients (PEC/PNEC) are tabulated below

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	3	4.6	**0.65**
Q - Ocean	0.3	4.6	**0.065**

The notified chemical is not expected to pose a risk to the environment when used as proposed in cosmetic and personal care products, as the risk quotients are less than one even under the hypothetical worst case assumption that the total imported quantity will be discharged unchanged to receiving waters from sewage treatment plants.

The risk quotient for freshwater environments would exceed 1 if the import quantity reached 7.7 tonnes.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The following risk phrases apply to the notified chemical:

Xi; R38 Irritating to skin
Xi; R41 Risk of serious damage to eyes

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Irritant	2	Causes skin irritation
Irreversible effects	1	Causes serious eye damage
Acute hazards to the aquatic environment	Acute 1	Very toxic to aquatic life

Human health risk assessment

Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of cosmetic production and transport workers.

Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of beauty care professionals. However, the risks from using spray products containing the notified chemical cannot be ruled out and are therefore not supported.

When used in dermal and oral care products as proposed, the notified chemical is not considered to pose an unacceptable risk to public health. However, the risks from using spray products containing the notified chemical cannot be ruled out and are therefore not supported.

Environmental risk assessment

On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment. It is noted that the risk quotient for freshwater environments would exceed 1 if the import quantity reached 7.7 tonnes.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- Safe Work Australia should consider the following hazard classification for the notified chemical:
  - Xi; R38 Irritating to skin
  - Xi; R41 Risk of serious damage to eyes

- Use the following cut-off concentrations for products/mixtures containing the notified chemical:
  - Conc > 20%: R38, R41
  - 10% < Conc < 20%: R41
  - 5% < Conc ≤ 10%; R36

- Use the following safety phrases for products/mixtures containing the notified chemical:
  - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Material Safety Data Sheet

- The MSDS provided by the notifier should be amended as follows:
  - The following risk phrase should be added: R38 – irritating to skin.

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced;
  - local exhaust ventilation for weighing and transfer activities

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced;
  - Avoid contact with skin and eyes
  - Avoid generation of aerosols

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - Impervious gloves and coveralls
  - Eye protection e.g. Safety glasses/face mask

- Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]

workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Public Health

- The following measures should be taken to minimise public exposure to the notified chemical:
  - the notified chemical should not be in spray products for consumer/domestic use.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, collection and subsequent safe disposal

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
  - the notified chemical is imported for use in spray products.
  - the notified chemical is used in cosmetic and personal care products at a concentration > 0.4%, unless being used as an active ingredient.
  - the notified chemical is used as an active ingredient in cosmetic and personal care products at a concentration > 0.8%.

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a preservative or active ingredient in cosmetic and personal care products or is likely to change significantly;
  - the amount of chemical being introduced has increased from 5 tonnes or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet
The MSDS of the imported product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 50.5 to 58.0 ºC

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Metal block method
Test Facility Huntingdon Life Sciences Ltd (2001a)

Boiling Point Decomposes from 107ºC at 101.3 kPa

Method EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Differential scanning calorimetry
Test Facility Huntingdon Life Sciences Ltd (2001a)

Density 1110 kg/m^3 at 20ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Pycnometer method
Test Facility Huntingdon Life Sciences Ltd (2001a)

Vapour Pressure 5.45 x 10^{-7} kPa at 25ºC

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Vapour pressure balance
Test Facility Huntingdon Life Sciences Ltd (2001a)

Water Solubility > 247 g/L at 20ºC

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method. Higher concentrations could not be tested because of difficult handling conditions and problems with separation of undissolved material.
Test Facility Huntingdon Life Sciences Ltd (2001a)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$*
4	25	> 1 year
7	25	57 days
9	25	34 hours

Remarks Hydrolysis followed pseudo first order kinetics. Hydrolysis was sufficiently rapid at pH 9 to allow direct calculation of the rate constant from measurements at 25ºC.
Test Facility Huntingdon Life Sciences Ltd (2000a, 2001b)

Partition Coefficient (n-octanol/water) log P_{ow} = 1.43 at 20ºC

Method OECD TG 107 Partition Coefficient (n-octanol/water): Shake Flask Method.
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Shake Flask Method with quantitation by HPLC (UV)
Test Facility Huntingdon Life Sciences Ltd (2001a)

Surface Tension 25.43 mN/m at 19ºC

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension. OECD harmonised ring method
Remarks	Concentration: 1 g/L aqueous solution Determined using surface tension/torsion balance
Test Facility	Huntingdon Life Sciences Ltd (2001a)

Adsorption/Desorption – screening test log K_{oc} = 1.76 at 20°C

Method	Calculation
Remarks	The result was obtained by calculation, based on the partition coefficient. The much higher value of 4.36 obtained by HPLC (OECD TG/94.75) was considered unreliable given the high water solubility, and is likely to be an artefact of the surface activity.
Test Facility	Huntingdon Life Sciences Ltd (2001a)

Dissociation Constant pK_a = 9.04 (α-amino), 12.48 (side chain)

Method	Not applicable
Remarks	The foregoing values are based on the amino groups in arginine (http://www.cem.msu.edu/~cem252/sp97/ch24/ch24aa.html accessed 19 February 2009) which is the most basic of all amino acids. The notified chemical is a hydrochloride salt that will be fully ionised under environmental conditions.

Particle Size

Method Sieve analysis

Range (μm)	*Mass (%)*
< 10	0
10-30	0.1
30-75	2.3
75-125	5.5
125-400	33.1
>400	59.0

Remarks	Inhalable fraction: < 8% Respirable fraction: 0%
Test Facility	Huntingdon Life Sciences Ltd (2001a)

Flammability Not highly flammable

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Test Facility	Huntingdon (2001a)

Autoignition Temperature Does not self ignite up to temperatures of 400ºC

Method	EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Test Facility	Huntingdon Life Sciences Ltd (2001a)

Explosive Properties Not explosive

Method	EC Directive 92/69/EEC A.14 Explosive Properties.
Test Facility	Huntingdon Life Sciences Ltd (2001a)

Oxidizing Properties Not oxidising

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Test Facility	Huntingdon Life Sciences Ltd (2001a)

Heavy Metal Analysis

Pb < 0.5 ppm
Cd < 0.1 ppm
Hg < 0.5 ppm
As < 5 ppm

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	Digestion of test substance with acid, followed by determination of metal content using ICP-MS
Test Facility	Universitat de Barcelona (2000)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (90.1%)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 96/54/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Sprague-Dawley CD
Vehicle	1% w/v aqueous methylcellulose
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	Signs of systemic toxicity in female animals comprised piloerection, increased salivation, waddling/unsteady gait, hunched posture and soiled fur. Signs of systemic toxicity in male animals comprised piloerection, increased salivation and hunched posture. All signs of systemic toxicity had resolved by 3 days after dosing for male animals or 4 days for female animals. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	There were no remarkable necropsy findings.
Remarks - Results	None
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Huntingdon Life Sciences Ltd (2000b)

B.2. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (20.3%)
METHOD	EC Directive 92/69/EEC B.1 Acute Toxicity (Oral).
Species/Strain	Rat/ Sprague-Dawley CD
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	Signs of systemic toxicity in all animals was confined to piloerection, with complete recovery seen by day 2. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	There were no remarkable necropsy findings.
Remarks - Results	None
CONCLUSION	The notified chemical is of low toxicity via the oral route.

TEST FACILITY Huntingdon Life Sciences Ltd (1995a)

B.3. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical (90.1%)

METHOD OECD TG 402 Acute Dermal Toxicity.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).

Species/Strain	Rat/ Sprague-Dawley CD
Vehicle	1% w/v aqueous methylcellulose
Type of dressing	Occlusive
Remarks - Method	Readings for humidity and temperature were not taken on 2 occasions, this was not expected to have affected the validity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Well-defined erythema and oedema was notable in all rats following removal of the dressings on day 2 and persistent at this level throughout the following days before resolving in all but 2 instances by day 9. In these two rats the erythema and oedema persisted through to day 12 or 14 before resolving. Associated with the erythema and oedema was blanching of the skin, desquamation, localised spots, scabbing and thickening of the skin.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity. All animals showed an expected gain in bodyweight during the study.
Effects in Organs	There were no remarkable necropsy findings, with the exception of scabbing on the dose sites of 3 animals and thickened tissues on 1.
Remarks - Results	None

CONCLUSION The notified chemical is of low toxicity via the dermal route.

TEST FACILITY Huntingdon Life Sciences Ltd (2000c)

B.4. Acute toxicity – inhalation

TEST SUBSTANCE Ethyl lauroyl arginate HCl (0.63% in propellants)

METHOD OECD TG 403 Acute Inhalation Toxicity – Limit Test.
EC Directive 92/69/EEC, 93/21/EEC B.2 Acute Toxicity (Inhalation) – Limit Test.

Species/Strain	Rat/Wistar/Hsd Cpb:WU (SPF)
Vehicle	Ethanol with propellants (solubility of the test substance in ethanol was not provided)
Method of Exposure	Nose-only exposure.
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	4.7 μm Mass Median Aerodynamic Diameter. The particle size and distribution seemed constant over the dosing period, with the aerosol generated found to be in the highly respirable range and a steady state concentration obtained during exposure.
Remarks - Method	GLP compliant. Two-week observation period post exposure. Air was supplied at a constant flow rate of 20 litres/minute.

The concentrations in the breathing zone of the rats were determined based on the non-volatile LAE and the most volatile constituents (the propellants). The actual concentration of the test substance was determined by back calculation from the analytical concentrations of propane and butane, as this was considered to best represent the amount of sprayed formulation.
The mean recovery of the propellants (vapour) was 68% and the active ingredient (LAE in ethanol as an aerosol) was 14%. The lower recovery of aerosolised LAE is likely to be related to the settling of the larger particles containing ethanol, isobutane and the non-volatile LAE.
The test conditions appear to have fulfilled the requirements for steady-state concentration, respirability of particles and the limit concentration tested.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <mg/m³>*		*Mortality*
		Nominal	*Actual*	
1	5/sex	0	0	0
2	5/sex	42000	5883*	0
			28150**	

*Concentration of test substance calculated based on measured aerosol fraction in the breathing zone area, which is equivalent to ~37.3 mg/m^3/4 hours notified chemical
**Concentration of the test substance calculated based on measured volatile fraction.

LC50	>5883 mg/m^3/4 hours (worst case value based on back calculation from measured aerosol in breathing zone. This is equivalent to >37.3 mg/m^3/4 hours notified chemical.
Signs of Toxicity	Treated rats showed bradypnea, laboured breathing patterns, irregular breathing patterns, high-legged gait, and piloerection. Most of these effects were resolved by day 1, with the exception of irregular breathing patterns and piloerection, which were resolved by day 4 or 5 after exposure. Rectal temperatures measured shortly following exposure were statistically significantly lower in the treated groups compared to the control, which was considered indicative of hypothermia. Body weight and body weight gains were reduced in most male treated groups compared to controls, however, these were not considered to be toxicologically significant.
Effects in Organs	There were no findings of toxicological significance.
Remarks - Results	The test report indicates that the test substance may have mild respiratory irritation potential if exposure to the aerosol is sufficiently high. Much of the non-volatile LAE appears to have been lost prior to reaching the breathing zone and as such, exposure is likely to be difficult to assess.

CONCLUSION The tested substance (0.63% notified chemical) is of low toxicity via inhalation.

TEST FACILITY Bayer (2006)

B.5. Irritation – skin

TEST SUBSTANCE Notified chemical (90.1%)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Water

Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	The animals were maintained at a lower temperature (15-21°C) than specified in the guideline (17-23°C).

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.7	1.0	2.0	2.0	> 14 days	1.0
Oedema	0.0	0.0	0.3	1.0	< 14 days	0.0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	There were no signs of systemic toxicity and no unexpected changes to body weight. Desquamation was observed in all three animals from day 7, which resolved in one animal but was still present in two animals at day 14. Persistent erythema (score 1) was also observed in one animal at the end of the study period.
CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	Research Toxicology Centre (2000)

B.6. Irritation – eye (1)

TEST SUBSTANCE	Notified chemical (99%) Summary of study given in SCCP, 2008.
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand Albino
Number of Animals	3 males
Vehicle	Not specified
Observation Period	21 days
Remarks - Method	Does not appear to have any significant protocol deviations.

RESULTS

Remarks - Results	After one hour, the following effects were observed in all animals: Redness of the conjuctiva with some hyperaemic blood vessels in all animals, swelling with the eyelids closed, scattered or diffuse corneal opacity that obscured the iris. After 72 hours, the following effects were observed in all animals: Redness of the conjuctiva, corneal opacity, no discernible iris due to the opacity, swelling with lids closed, lacrimation, moistening of the eye lids and the fur. After 21 days, the following effects were observed in all animals: Diffuse, crimson redness of the conjunctiva with individual vessels not easily discernible, swelling with lids half closed, tissue growth in the cornea. After 21 days: Two animals displayed lacrimation with moistening of lids and fur. Corneal opacity was noted in one animal, whilst the other two showed areas of corneal opacity with no visible iris. The mean scores for each type of lesion (calculated on the basis of scores at 24, 48, and 72 hours) for the 3 animals were as follows:

Corneal opacity: 4.0
Iridial lesions: No quantification possible
Hyperaemia: 3.0
Oedema: 4.0

CONCLUSION The notified chemical causes serious damage to the eyes.

TEST FACILITY Centro de Investigación y Desarrollo Aplicado, S.A.L. (1997)

B.7. Irritation – eye (2)

TEST SUBSTANCE Notified chemical (20.4%)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	1 female
Vehicle	Propylene glycol
Observation Period	48 hours
Remarks - Method	No significant protocol deviations. The test animal was killed after 48 hours due to sloughing of the nictitating and conjunctival membranes. After consideration of the ocular responses produced in the first treated animal, no further animals were treated.

RESULTS

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1			
Conjunctiva: redness	2	2	48 hours	2
Conjunctiva: chemosis	2	3	48 hours	2
Conjunctiva: discharge	2.5	3	48 hours	2
Corneal opacity	2.5	3	48 hours	3
Iridial inflammation	1	1	48 hours	1

*Calculated on the basis of the scores at 24 and 48 hours.

Remarks - Results Diffuse corneal opacity was noted at the 1 hour observation with translucent corneal opacity at the 24 hour observation and opalescent corneal opacity at the 48 hour observation. Sloughing of the cornea was noted at the 24 and 48 hour observations.
Petechial haemorrhage of the upper conjunctival membrane was noted at the 1, 24 and 48 hour observations with sloughing of the conjunctivae at the 48 hour observation.

CONCLUSION The notified chemical is severely irritating to the eye at 20.4% concentration.

TEST FACILITY Safepharm (1995a)

B.8. Irritation – eye (3)

TEST SUBSTANCE Notified chemical (20.4%)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	1

Vehicle	Water dispersed
Observation Period	24 hours
Remarks - Method	No significant protocol deviations. The test animal was killed after 24 hours due to sloughing of the lower conjunctival membrane. After consideration of the ocular responses produced in the first treated animal, no further animals were treated.

RESULTS

Lesion	*Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2	2	24 hours	2
Conjunctiva: chemosis	2	2	24 hours	2
Conjunctiva: discharge	2	3	24 hours	2
Corneal opacity	2	2	24 hours	2
Iridial inflammation	1	1	24 hours	1

*As the animal was killed after the 24-hour observation the values are based on this observation only.

Remarks - Results	Diffuse corneal opacity was noted at the 1-hour observation with translucent corneal opacity at the 24-hour observation. Sloughing of the cornea was noted at the 1 and 24 hour observations. Sloughing of the lower conjunctival membrane was noted at the 24-hour observation.

CONCLUSION	The notified chemical is severely irritating to the eye at 20.4% concentration.
TEST FACILITY	Safepharm (1996)

B.9. Irritation – eye (4)

TEST SUBSTANCE	Notified chemical (0.4%)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 male
Observation Period	14 days
Remarks - Method	The temperature and relative humidity in the animal housing was maintained at approximately 18-25°C and 55-80% respectively. This deviation in the protocol was considered to not effect the results of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.3	1.3	0.3	2	< 14 days	0
Conjunctiva: chemosis	0.7	1	0	3	< 7 days	0
Conjunctiva: discharge					Not measured	
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	1	< 24 hours	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	Conjunctival discharge was not scored although it was noted in all animals at the 1-hour observation and in 1 animal at the 24 hour observation. Lesions in the iris were noted in two animals at the 1-hour observation.

Redness of the conjunctivae was noted in all animals at the 1 and 24 hour observations and in two animals at the 48 and 72 hour observations and 1 animal only at the 7 day observation with no effects seen in any of the animals at the 14 day observation.
Oedema of the conjunctivae (chemosis) was present in all animals at the 1-hour observation and in 2 animals at the 24 and 48-hour observations with only one animal showing symptoms at the 72-hour observation and no signs seen at latter observations.

CONCLUSION The notified chemical is slightly irritating to the eye at 0.4% concentration.

TEST FACILITY Centro de Investigación y Desarrollo Aplicado, S.A.L. (2000a)

B.10. Irritation – eye (5)

TEST SUBSTANCE Notified chemical (0.04%)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain Rabbit/New Zealand White
Number of Animals 3 male
Observation Period 7 days
Remarks - Method The temperature and relative humidity in the animal housing was maintained at approximately 18-25°C and 55-80% respectively. This deviation in the protocol was considered to not affect the results of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1	0.3	0.3	2	< 7 days	0
Conjunctiva: chemosis	0	0	0.3	1	< 48 hours	0
Conjunctiva: discharge					Not measured	
Corneal opacity	0.3	0	0	1	< 48 hours	0
Iridial inflammation	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results Conjunctival discharge was not scored although it was noted in 2 animals at the 1-hour observation.
Redness of the conjunctivae was noted in all animals at the 1-hour observation and in 2 animals at the 24 and 48-hour observations and 1 animal only at the 72-hour observation with no effects seen in any of the animals at the 7-day observation.
Oedema of the conjunctivae (chemosis) was present in 2 animals at the 1-hour observation and in 1 animal at the 24-hour observation and no signs seen at latter observations.

CONCLUSION The notified chemical is slightly irritating to the eye at 0.04% concentration.

TEST FACILITY Centro de Investigación y Desarrollo Aplicado, S.A.L. (2000b)

B.11. Irritation – eye (6)

TEST SUBSTANCE Notified chemical (0.03%)

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 male
Observation Period: 7 days
Remarks - Method: The temperature and relative humidity in the animal housing was maintained at approximately 18-25°C and 55-80% respectively. This deviation in the protocol was considered to not effect the results of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	0.7	1	< 7 days	0
Conjunctiva: chemosis	0	0.3	0	1	< 48 hours	0
Conjunctiva: discharge					Not measured	
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results: Conjunctival discharge was not scored although it was noted in 1 animal at the 1-hour observation.
Redness of the conjunctivae was noted in all animals at the 1-hour observation and in 1 animal at the 24 and 72-hour observations but not at the 48-hour observation with no effects seen in any of the animals at the 7-day observation.
Oedema of the conjunctivae (chemosis) was present in 1 animal at the 1-hour observation and in a different animal at the 24-hour observation and no signs seen at latter observations.

CONCLUSION: The notified chemical is slightly irritating to the eye at 0.03% concentration.

TEST FACILITY: Centro de Investigación y Desarrollo Aplicado, S.A.L. (2000c)

B.12. Irritation – eye (7)

TEST SUBSTANCE: Notified chemical (0.02%)

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 male
Observation Period: 7 days
Remarks - Method: The temperature and relative humidity in the animal housing was maintained at approximately 18-25°C and 55-80% respectively. This deviation in the protocol was considered to not effect the results of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.7	0	0	1	< 7 days	0
Conjunctiva: chemosis	0	0.3	0	1	< 48 hours	0

Conjunctiva: discharge				Not measured		
Corneal opacity	0	0.3	0	1	< 48 hours	0
Iridial inflammation	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results

Redness of the conjunctivae was noted in all animals at the 1-hour observation and in 1 animal at the 24 and 72-hour observations but not at the 48-hour observation with no effects seen in any of the animals at the 7-day observation.
Oedema of the conjunctivae (chemosis) was present in 1 animal at the 1 and 24-hour observations with no signs seen at latter observations.
Scattered or diffuse corneal opacity was noted in one animal at the 24-hour observation.

CONCLUSION

The notified chemical is slightly irritating to the eye at 0.02% concentration.

TEST FACILITY

Centro de Investigación y Desarrollo Aplicado, S.A.L. (2000d)

B.13. Irritation – eye (8)

TEST SUBSTANCE

Notified chemical (0.8%)

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 male
Observation Period: 14 days
Remarks - Method: No significant protocol deviations.
The temperature in the animal housing was maintained at 19-25°C. This deviation in the protocol was considered to not effect the results of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.3	1.3	1	2	< 14 days	0
Conjunctiva: chemosis	0	0.7	0.3	2	< 72 hours	0
Conjunctiva: discharge				Not measured		
Corneal opacity	0	0.3	0	1	< 48 hours	0
Iridial inflammation	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results

All animals showed an expected gain in bodyweight during the study. However, one animal showed a 20 g loss in weight in the first 24 hours after administration.
Conjunctival discharge was not scored although it was noted in all animals at the 1-hour observation and in 1 animal at the 24 and 48-hour observations.
Redness of the conjunctivae was noted in all animals at the 1 and 24 and 48-hour observations and in 2 animals at the 72-hour observation and 1 animal only at the 7-day observation with no effects seen in any of the animals at the 14-day observation.
Oedema of the conjunctivae (chemosis) was present in 2 animals at the 1-hour observation and in 2 animals at the 24-hour observation with only one animal showing symptoms at the 48-hour observation and no signs seen at latter observations.

Scattered or diffuse corneal opacity was noted in one animal at the 24-hour observation.

CONCLUSION The notified chemical is slightly irritating to the eye at 0.8% concentration.

TEST FACILITY RCC CIDA S.A. (2006)

B.14. Skin sensitisation (1)

TEST SUBSTANCE Notified chemical in aqueous solution (20 - 20.4%). The purity of the test material was not analysed prior to the study therefore the final administered concentrations are unknown.

METHOD OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test.
EC Directive 92/69/EEC B.6 Skin sensitisation

Species/Strain Guinea pig/Dunkin Hartley

PRELIMINARY STUDY
Maximum Non-irritating Concentration:
intradermal: < 1%
topical: 10%
Maximum concentration to cause mild-moderate irritation:
intradermal: 1%
topical: 50%

MAIN STUDY

Number of Animals Test Group: 10 Control Group: 5

INDUCTION PHASE
Induction Concentration:
intradermal: 1%
topical: 50%

CHALLENGE PHASE

1st challenge topical: 25%, 50%

Remarks - Method There were no significant protocol deviations.

RESULTS

Remarks - Results

Preliminary study
Intradermal: moderate-severe erythema was observed at the 24 and 48 observations in one animal treated with 1% test material. This reduced to well-defined or slight erythema by day 7. Severe erythema with necrosis was noted at the 24 and 48 hour observation in an animal dosed at 5%. Severe erythema and eschar was observed in the same animal at 72 hours, which persisted to the day 7 observation. Oedema scores are not known, as it was not evaluated as a part of this test. No tests were performed at concentrations below 1%.

Topical: Three out of 4 animals exposed to 25% test material showed slight erythema after one hour, which resolved by the 24 hour observation. One animal appeared to show no skin reaction initially, however desquamation of the treated site was observed 48 hours after patch removal. Two animals that were exposed for a 24 hour period to 50% test material experienced mild erythema that cleared by 24 hours. Animals that were exposed for 48 hours to concentrations ≥ 50% exhibited desquamation, slight oedema as well as an "adverse reaction" at the test site that "prevented evaluation of erythema". No subsequent observations were made to assess the severity or reversibility of these adverse skin reactions.

Main study
Intradermal induction: All ten animals showed well-defined or moderate-

severe erythema at 24 and 48 hours after exposure to 1% test material. Slight erythema was noted in two control animals at the 24 hour observation.

Topical induction: Slight erythema was noted at all induction sites in all ten animals at the 1 hour observation and two animals at the 24 hour observation. There were no skin reactions in the control group.

Topical Challenge: No skin reactions were noted at any challenge site in any animal at either concentration.

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.

TEST FACILITY Safepharm (1995b)

B.15. Skin sensitisation (2)

TEST SUBSTANCE Notified chemical as solid powder (LAE)

METHOD OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test.
EC Directive 96/54/EC B.6 Skin Sensitisation - Guinea Pig Maximisation Test.

Species/Strain Guinea pig/Dunkin Hartley

PRELIMINARY STUDY
Maximum Non-irritating Concentration:
intradermal: < 0.5%
topical: 10%
Maximum concentration to cause mild-moderate irritation:
intradermal: < 0.5%
topical: 50%

MAIN STUDY

Number of Animals Test Group: 10 Control Group: 5

INDUCTION PHASE
Induction Concentration:
intradermal: 0.1%
topical: 20%

CHALLENGE PHASE

1st challenge topical: 5%

Remarks - Method In the preliminary study, the test material was delivered with water as the vehicle. In the main study, the test material was delivered as a mixture of FCA, paraffin oil, an emulsifier and killed mycobacteria to enhance the potential of delayed contact hypersensitivity.

RESULTS

Remarks - Results

Preliminary study
Intradermal: application of 0.5 and 1% test substance at caused discolouration of the treated sites in 2/2 animals tested. Higher doses (5-50%) resulted in skin necrosis on all animals. No tests were performed at concentrations below 0.5%.

Topical: Two of two animals given 20 and 50% topical doses exhibited slight or patchy erythema at 24 hours and this completely resolved by 48 hours.

Main study
Intradermal induction: Well-defined erythema was apparent in control animals following an injection of FCA emulsion alone and FCA/vehicle and in the test group injected with FCA/0.1% test substance. No reaction was observed in the control group treated with the vehicle alone. In the

treatment group, 5/10 animals injected with 0.1% test substance showed slight erythema 24 hours after injection.

Topical induction: No reaction was observed around the injection sites following 48 hours topical exposure to 20% test substance (test group) or vehicle alone (control group).

Topical challenge: No response was observed in any animal of both control group and test group following 24 hours exposure to 5% test substance.

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.

TEST FACILITY Research Toxicology Centre (2001)

B.16. Repeat dose toxicity (1)

TEST SUBSTANCE LAE (90.1% notified chemical)

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain Rat/Han Wistar
Route of Administration Oral –diet
Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Remarks - Method There was no post-exposure observation period following the 28 days. No other significant protocol deviations.

RESULTS

Group	*Dose of notified chemical (mg/kg bw/day) (M/F)*	*Number and Sex of Animals*	*Mortality*
control	0	5 per sex	0
low dose (25000 ppm)	2120/2143	5 per sex	0
mid dose (37500 ppm)	3098/2999	5 per sex	0
high dose (50000 ppm)	3850/4182	5 per sex	0

Mortality and Time to Death
No mortalities occurred during the study.

Clinical Observations
Piloerection and an ungroomed coat were observed in all females treated with 4182 mg/kg bw/day and 2 females treated with 2999 mg/kg bw/day. Salivation was observed in all females and the majority of males in the high dose group. Brown staining of the muzzle was observed in animals from each group treated with the test substance.
Decreased weight gains in treated males were observed in a dose-dependent manner across all treatment groups in week 1. Weight gain over the remainder of the study increased but did not reach the same rate as controls. In females, weight gain was increased in the groups treated with 2999 and 2143 mg/kg bw/day. Weight gain in the group treated with 4182 mg/kg bw/day was markedly lower until day 7, after which, it was comparable to controls.

Food consumption was decreased in a dose-dependent manner in all groups of treated animals during the first week of treatment. For the remainder of the study, food consumption in treated animals remained lower compared to control animals, with the decrease in food consumption in males inversely related to the dose.
During the first week of the study, food conversion efficiency was unable to be calculated for males treated with 3850 mg/kg bw/day due to weight loss observed in that group. In the first week, food conversion efficiency was markedly low for females treated with 4182 mg/kg bw/day and slightly low for males receiving

3098 mg/kg bw/day. For the remainder of the study, food conversion efficiencies were similar to or greater than the control group, with particularly high food conversion efficiency observed in females treated with 4182 mg/kg bw/day.

Laboratory Findings – Clinical Chemistry, Haematology

Mean cell haemoglobin concentration and basophil concentration was lower in males treated with 3850 mg/kg bw/day. Heamoglobin, mean cell haemoglobin and mean cell volume values were elevated in females treated with 4182 mg/kg bw/day.

Total bilirubin levels were observed in males treated with 3850 mg/kg bw/day. Decreased levels in calcium, total protein and albumin were observed in males treated with 3850 and 3098 mg/kg bw/day. Males treated with 2120 mg/kg bw/day also had decreased total protein levels.

Females treated with 4182 mg/kg bw/day displayed increased alkaline phosphate, alanine amino-transferase and aspartate amino-transferase levels and females treated with 2999 mg/kg bw/day also showed elevated alanine amino-transferase levels.

Effects in Organs

Effects, such as, dilated kidney, functate foci of the thymus, aerated fluid in the trachea, partially collapsed lung, prematurely inflated lung, enlarged parotid sublingual gland, misshapen spleen and fluid distention in the uterus were found in rats in all study groups, including control animals.

In comparison to controls, statistically significant decreases in the absolute weight of the spleen of males treated with 3850 and 3098 mg/kg bw/day were observed. Statistically significant increases in relative brain weights of all males treated with the notified chemical were also observed.

Remarks – Results

The notified chemical was found to be unpalatable in the diet during the first week of the study demonstrated by the decreased food consumption in animals in the high and mid dose groups when compared to animals in the control groups. However, tolerance to diet containing the test substance improved after week 1 even though bodyweights of treated males remained lower than controls throughout the study. Conversely, bodyweights of females treated with the notified chemical at 2999 and 2143 mg/kg bw/day were increased compared to controls over the duration of the study.

The statistically significant increase in haematological parameters in females treated with 4182 mg/kg bw/day were not considered to be related to treatment given there were no other associated effects.

Low protein and albumin concentrations in male rats treated with 3850 and 3098 mg/kg bw/day and high enzyme concentrations in females rats treated with 3850 mg/kg bw/day may be indicative of liver effects. However, no weight changes or macroscopic findings in the liver were established at termination of the study. Therefore, these changes were considered not to be of toxicological significance.

Various effects in the organs were observed at termination of the study. However, these were considered not related to treatment given that similar effects were observed in control animals and the incidence was not dose-dependent.

The decreased absolute weight of spleens of males treated with 3850 and 3098 mg/kg bw/day was considered a result of the lower bodyweights of males at those doses. This was supported by a lack of macroscopic findings in the spleen at necropsy. An increase in relative brain weight was also observed in males treated at all dose levels. However, given the absolute brain weights were comparable to controls, the higher relative brain weights in treated males was considered to be a result of lower average bodyweights in those animals.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) of the notified chemical was established as 3850 mg/kg bw/day (males) and 4182 mg/kg bw/day (females) by the study author, based on the lack of treatment-related adverse effects at that dose level.

TEST FACILITY Huntingdon Life Sciences Ltd (2000d)

B.17. Repeat dose toxicity (2)

TEST SUBSTANCE	Notified chemical (20.3%)
METHOD	Equivalent to OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Crl:CD BR
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Remarks - Method	There was no post-exposure observation period following the 28 days. Neurological assessments were not performed. No other significant protocol deviations.

RESULTS

Group	*Dose of notified chemical mg/kg bw/day (M/F)*	*Number and Sex of Animals*	*Mortality*
control	0	5 per sex	0
low dose (3200 ppm)	68/71	5 per sex	0
mid dose (12800 ppm)	283/284	5 per sex	0
high dose (50000 ppm)	1070/1187	5 per sex	0

Mortality and Time to Death
No mortalities occurred during the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Mean corpuscular haemoglobin levels in male rats treated with 1070 mg/kg bw/day were elevated compared to the control group animals.

Effects in Organs
Enlarged cervical lymph nodes were observed in 16/30 animals in each sex at each dose level as well as the control group.

Increases in relative liver weights were observed in males treated with 1070 and 283 mg/kg bw/day.

Remarks – Results
The observation of enlarged cervical lymph nodes in over 50% of the test animals was not considered to be related to treatment given that animals in the control group also displayed enlarged cervical lymph nodes.

Modest, treatment-related increases (16% and 6%) were observed in liver weights in males treated with 1070 and 283 mg/kg bw/day respectively. However, the increases were not dose-dependent and no significant histopathological correlates were found.

The increase in mean corpuscular haemoglobin levels in male rats treated with 1070 mg/kg bw/day was statistically significant but was not considered to be of toxicological significance given other haematological parameters were not significantly affected.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1070 mg/kg bw/day (males) and 1187 mg/kg bw/day (females) by the study authors, based on the absence of toxicologically significant findings at this dose level. However, NICNAS considers 283 mg/kg bw/day to be the NOAEL, considering the increased liver weights (16% increase) and mean corpuscular haemoglobin levels in males at the highest dose level of 1070 mg/kg bw/day.

TEST FACILITY Huntingdon Life Sciences Ltd (1995b)

B.18. Repeat dose toxicity (3)

TEST SUBSTANCE	Notified chemical (20.2%)
METHOD	OECD TG 408 Repeated Dose 13 week Oral Toxicity Study in Rodents.
Species/Strain	Rat Crl: CD(SD)BR
Route of Administration	Oral –diet
Exposure Information	Total exposure days: 13 weeks Dose regimen: 7 days per week Post-exposure observation period: none
Remarks - Method	There was no post-exposure observation period following the 13 weeks. No other significant protocol deviations.

RESULTS

Group	*Dose of notified chemical (mg/kg bw/day) (Male/Female)*	*Number and Sex of Animals*	*Mortality*
control	0	10/sex	1
low dose (3200 ppm)	44/53	10/sex	0
mid dose (12800 ppm)	183/216	10/sex	0
high dose (50000 ppm)	671/793	10/sex	0

Mortality and Time to Death

One male animal in the control group died during the first week. Post mortem examination revealed a ruptured liver as the likely cause of death.

Clinical Observations

Incidental hairloss was seen in all treatment and control groups with the number of animals affected slightly higher in the mid and high dose although no increase in the severity was seen at higher doses. Further isolated clinical signs seen in the animals include staining of the muzzle, scabbing, pale extremities and teeth.
Overall mean bodyweight gains for all treated male animals were similar to the concurrent controls. The mean bodyweight gain for female animals was significantly ($p<0.01$) lower in the 216 and 793 mg/kg bw/day dose groups, however no dose response was seen.

Food consumption in treated animals was comparable to control animals. Food conversion in treated female animals was found to be marginally inferior to controls although no dose response was present. No difference in the food conversion for treated male animals in comparison to the controls was found. Water consumption was higher for males in the 671 mg/kg bw/day dose group and lower for females in the 216 mg/kg bw/day dose group when compared to controls.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There was a slight statistically significant decrease in the total white blood cell counts for female animals in the 216 and 793 mg/kg bw/day dose groups. However, as there was not consistency in the types of white blood cells contributing to the lower total cell count, the effects were considered by the study authors to be of uncertain toxicological importance.

A decrease in the ornithine carbamoyl transferase values was seen in all of the treated groups, however, there was no dose response and outliers affected the control values.

Male animals in the 671 mg/kg bw/day dose group showed an increase in mean urine volume, however, the increase was not statistically significant.

Effects in Organs

Effects, including but not limited to, aggregates of lymphocytes in the prostrate and lungs, vascular congestion in the lungs, scattered fat deposition and centrilobular hepatocyte vacuolation in the liver, cortical tubular basophilia and (cortico) medullary mineralisation in the kidneys, prominent lymph follicles in the caecum and colon and fluid distension and luminal dilation in the uterus, exocrine atrophy in the pancreas were found in rats in all study groups, including control animals.

In comparison to controls, there was a slightly higher mean liver weight in females treated with 793 mg/kg bw/day. An increase in liver weight can be associated with adaptive changes following treatment with xenobiotics.

Remarks – Results
The mean bodyweight gain was significantly lower for female animals treated with the test substance, however, no dose response relationship was seen and therefore the effects were not considered to be adverse.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 793 and 671 mg/kg bw/day notified chemical in female and male rats, respectively, in this study by the study authors, based on the lack of clear treatment-related adverse effects at that dose level.

TEST FACILITY Huntingdon Life Sciences Ltd (1996)

B.19. Repeat dose toxicity (4)

TEST SUBSTANCE	Notified chemical (89.4%)
METHOD	OECD TG 408 Repeated Dose 13 week Oral Toxicity Study in Rodents.
Species/Strain	Rat Han Wistar
Route of Administration	Oral –diet
Exposure Information	Total exposure days: 13 weeks Dose regimen: 7 days per week Post-exposure observation period: none
Remarks - Method	There was no post-exposure observation period following the 13 weeks. No other significant protocol deviations.

RESULTS

Group	*Dose of notified chemical (mg/kg bw/day) (Male/Female)*	*Number and Sex of Animals*	*Mortality*
control	0	20/sex	0
low dose (5000 ppm)	343/398	20/sex	0
mid dose (15000 ppm)	1022/1150	20/sex	0
high dose (50000 ppm)	3320/3500	20/sex	0

Mortality and Time to Death
No mortalities occurred during the study.

Clinical Observations
Piloerection, an ungroomed coat and brown staining were observed in female animals treated with 3500 mg/kg bw/day and male animals treated with 3320 mg/kg bw/day. Brown coat staining was also observed in male and female animals treated with 1022 and 1150 mg/kg bw/day respectively.

Activity and rearing scores for male animals treated with 3320 mg/kg bw/day were markedly increased on several occasions during the first half of the treatment period. During a study of motor activity in week 12, no treatment related changes were seen and the initial increased activity levels may be a result of the decreased food consumption in these animals during the early parts of the study.

During week 1 of the study males treated with 3320 mg/kg bw/day showed a 16% decrease in bodyweight and females treated with 3500 mg/kg bw/day showed a 13% decrease in bodyweight. Bodyweight gains for male and female animals treated at 1022 and 1150 mg/kg bw/day respectively were also significantly lower than controls as were male animals treated with 343 mg/kg bw/day. Weight gain over the remainder of the study increased but did not reach the same amount as controls. Food consumption was markedly lower 33 and 39% of the control values for male and female animals respectively in the 3320 mg/kg bw/day and 3500 mg/kg bw/day dose groups but increased to 79 and 78% of control values by the end of the study. Food consumption was also lower in all animals in the mid dose groups and male animals in the low dose group. During the first

week of the study, food conversion efficiency was unable to be calculated for males and females treated with 3320 and 3500 mg/kg bw/day respectively due to weight loss observed in those groups. For the remainder of the study, food conversion efficiencies were similar to or slightly higher than the control group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Male animals treated with and 3320 mg/kg bw/day showed an increase in mean cell haemoglobin ($p < 0.01$), mean cell haemoglobin concentration ($p < 0.05$), mean cell volume ($p < 0.05$) and chloride levels ($p < 0.05$), and a decrease in the total and differential leucocyte count ($p < 0.01$), lymphocyte count ($p < 0.05$), glucose concentrations ($p < 0.01$), total protein concentration ($p < 0.05$), albumin concentration ($p < 0.01$) and total protein concentration ($p < 0.01$). Female animals treated with and 3500 mg/kg bw/day showed an increase in alkaline phosphatase ($p < 0.01$) and a decrease in total cholesterol concentration ($p < 0.05$).

The differences in the haematological parameters above were only seen in the high dose groups and no dose response was observed in many cases.

A dose response relationship with statistically significant decreases across all treatment levels in male animals was seen for gamma-glutamyl transferase activity, however, this was primarily due to two outliers in the control sample and therefore not indicative of toxicity in the test substance. Significant decreases ($p < 0.05$) in the calcium concentration for males animals treated with 1022 and 3320 mg/kg bw/day were also seen.

Significant decreases were seen in the glucose concentrations in treated female animals although no dose response relationship was present. Female animals in the 1150 and 3500 mg/kg bw/day treatment groups also showed a significant decrease in the alanine amino-transferase activity.

Male animals treated at 1022 and 3320 mg/kg bw/day showed a dose related decrease in urine pH values.

Effects in Organs

There were a number of statistically significant organ weight decreases many of which may have been due to the decrease in the bodyweight of the treated animals.

Effects seen in the stomach of the test animals included parakeratosis, which was seen in the majority (13/20) of male and female animals in the 3320 and 3500 mg/kg bw/day dose groups and 1 female animal treated with 1150 mg/kg bw/day. In the nonglandular region of the stomach ulceration was seen in 1 male animal in each of the 1022 and 3320 mg/kg bw/day treatment groups and 2 female animals in the 3500 mg/kg bw/day treatment group. Erosion was seen in 3 female animals and epithelial hyperplasia in 1 female animal in the 3500 mg/kg bw/day dose group. Haemorrhage was seen in the glandular region of the stomach in 1 male rat in each of the 1022 and 3320 mg/kg bw/day treatment groups.

Remarks – Results

No recovery period observations were conducted and therefore the reversibility of the stomach effects could not be determined. The decrease in the bodyweights seen in treated animals is primarily due to the lower food consumption caused by the unpalatability of the test substance and was not therefore considered to be indicative of toxicity. The decrease in bodyweight may also have been responsible for the decreased weigh seen in many of the organs in the high dose groups.

Treatment related effects seen in the stomach may be due to irritant action of the test substance on the mucosal tissue.

Individual values for haematological parameters were within the historical range expected.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 343 and 398 mg/kg bw/day notified chemical), based on the adverse effects (stomach ulceration and haemorrhage) seen at higher doses.

TEST FACILITY Huntingdon Life Sciences Ltd (2001c)

B.20. Repeat dose toxicity (5)

TEST SUBSTANCE Notified chemical (88.2%)

METHOD	OECD TG 452 Chronic Toxicity Studies. EC Directive 88/302/EEC B.30 Chronic Toxicity Test.
Species/Strain	Crl: CD (SD) IGS BR rats
Route of Administration	Oral –diet
Exposure Information	Total exposure: 52 weeks Dose regimen: 7 days per week (continuous) Post-exposure observation period: None.
Vehicle	LAE was mixed in with a standard ground diet to the desired concentrations (prepared weekly).
Remarks - Method	No significant protocol deviations. During the study, the following observations were undertaken regularly: clinical condition, body weight, food consumption, ophthalmic examination, haematology (peripheral blood), blood chemistry, urinalysis, physical examination, and arena observations. In addition, grip strength and motor activity were tested towards the end of the study, together with blood samples being withdrawn from animals for toxicokinetic and bioanalytical investigations. Upon completion of the study, bone marrow smears were prepared and a full myelogram completed. Macroscopic examination, organ weight measurements, and histopathological examinations were performed.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*			*Mortality*
		Nominal ppm	*Actual mg/kg/day*	*Active Actual* mg/kg/day*	
I (control)	20M	0	0	0	1
	20F				0
II (low dose)	20M	2000	M: 106	M: 93.5	2
	20F		F: 131	F: 116	1
III (mid dose)	20M	6000	M: 307	M: 271	0
	20F		F: 393	F: 347	0
IV (high dose)	20M	18000	M: 907	M: 800	1
	20F		F: 1128	F: 995	1

* Values accounting for the purity of the test substance.

Mortality and Time to Death

There were six unscheduled deaths during the study. The deaths were not considered to be treatment related.

Toxicokinetics

Rate and extent of exposure to LAE and its metabolite LAS were measured during Week 52 of the study. The results indicate that the exposure to the notified chemical did not increase in a linear fashion with increasing dose.

Clinical Observations

Up to Week 13 high dose females and to a lesser extent mid dose females showed higher incidences of generalised brown fur staining and ungroomed coats than the controls.

At the high and mid dose levels both sexes showed lower body weight gain and some reductions in food consumption compared to controls at various stages throughout the treatment period. There were also some reductions in initial food conversion efficiency observed during week 1 that generally coincided with reduced body weight gain and food intake compared to controls (with the exception of mid dose females). This indicated that the initial lower gains were not solely due to lower food intake. After week 1 the food conversion efficiencies were similar to controls, indicating a connection between lower food intake and reduced body weight gain.

In Week 49, high dose males exhibited higher beam motor activity scores (high and low beam motor activity) compared to controls.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Blood chemistry

Higher mean urea values were observed in all groups of treated females compared to controls, with a dose relationship observed between the mid and high dose groups, but not the low and mid dose groups. There was also a statistically significant increase in the albumin/globulin ratio observed in high dose female animals during week 26 of treatment that was also dose related in nature. This was not considered to be of toxicological relevance given that the effect was not present during week 52 of treatment.

Some statistically significant changes in other blood chemistry parameters were observed, though these were not considered to be of toxicological relevance given their isolated nature and the absence of dose related trends in the values.

Urinalysis

There were no effects on urinalysis parameters that were considered to be treatment related.

Haematology peripheral

Statistically significant effects on peripheral blood cell parameters compared to controls were observed at all doses, particularly for the white blood cell parameters, though the effects were inconsistent.

In males at week 14, statistically significant decreases in monocytes and LUC were observed in animals treated with the high dose. At the mid dose treatment level (males) this was the case only for monocytes levels. In female animals statistically significant decreases in monocytes and LUC were observed at all doses. At the high dose there were also significant decreases in neutrophils and reticulocytes.

In males by week 26 there were statistically significant increases in MCHC and decreases in WBC counts at all dose levels (such decreases were mainly attributed to decreased differential cell counts of lymphocytes and LUC at all dose levels, whilst at the high dose, decreased differential monocyte counts also contributed). In females, statistically significant decreases in the total WBC count were observed at the mid and high dose levels (largely due to decreases in the differential cell counts of monocytes and LUC at all dose levels, and lymphocytes at mid dose levels).

In males by week 52 there were only statistically significant decreases in the total WBC count at the highest dose. This was associated with a statistically significant decrease in the differential cell counts of neutrophils only, however, at all doses there were statistically significant decreases observed in lymphocytes, monocytes and LUC. In female animals statistically significant changes were only observed at the high dose and these were only decreases in the differential cell counts of neutrophils, monocytes and LUC and an increase in MCHC.

Haematology bone marrow

There were no clear effects of treatment on the bone marrow, as they were only minor differences from control, not dose related, and inconsistent across sexes. As such, they were considered not to be associated with treatment.

Effects in Organs

Stomach effects

Macroscopically evident depressions on the epithelial aspect of the forestomach (oesophageal groove) were observed in 12/19 males and 9/19 females in the high dose groups, 5/20 males and 6/20 females at the mid dose groups, 1/18 males and 5/19 females in the low dose groups and 0/19 males and 2/19 females in the control groups.

These forestomach effects were broadly reflected by the histopathology observations. Lesions were observed at the non-glandular epithelium of the stomach in 9/19 males and 8/19 female animals treated with the high dose, including slight erosion, and minimal or slight ulceration, re-epithelialisation and hyperplasia. These lesions were accompanied by subepithelial/submucosal inflammation, subepithelial fibrosis and inflammation of the muscle and serosal layers. These changes were only statistically significant in comparison to controls for the high dose group, although similar forestomach lesions were also observed in the mid (4/20 males, 5/20

females) and low dose groups (1/18 males, 5/19 females).

Remarks – Results
Whilst there were treatment-related effects on white blood cell parameters in both sexes, there was no clear dose related trend, no associated bone marrow effects or histopathology related to the lymphoid tissues. As such, these effects were not considered to be of toxicological significance. In addition, the white blood cell changes may occur as a physiological response to the changes in the stomach and associated tissues.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 271 mg/kg bw/day of the notified chemical in males and 347 mg/kg bw/day of the notified chemical in females in this study, based on the statistically significant incidence of forestomach lesions at the high dose level.

TEST FACILITY Huntingdon Life Sciences Ltd (2005a)

B.21. Genotoxicity – bacteria (1)

TEST SUBSTANCE Notified chemical (89.4%)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation (first test), Pre-incubation procedure (second test)

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP2uvrA (pKM101)
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 1.5-150 µg/plate b) Without metabolic activation: 1.5-150 µg/plate
Vehicle	DMSO
Remarks - Method	Due to toxicity at higher concentrations in the range-finding test, the test was repeated at lower concentrations in the main study. No other significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 50-5000	150	> 150	Negative
Test 2		150	> 150	Negative
Present				
Test 1	≥ 50-5000	150	> 150	Negative
Test 2		150	> 150	Negative

Remarks - Results
Cytotoxicity was observed in all *Salmonella* strains at concentrations of 50 µg/plate and greater in the absence and presence of metabolic activation. Cytotoxicity was observed in *E. coli* WP2uvrA (pKM101) at concentrations of 150 µg/plate or greater in the absence and presence of metabolic activation.

No substantial increases in the number of revertant colonies were seen in any strain either in the presence or absence of metabolic activation.
The negative controls were within normal limits and the positive controls (Sodium azide, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, 9-aminoacridine, 2-nitrofluorene (-S9); 2-aminoanthracene, Benzo[a]pyrene (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Huntingdon Life Sciences Ltd (2001d)

B.22. Genotoxicity – bacteria (2)

TEST SUBSTANCE	20.3% LAE (notified chemical) 73.5% Propylene glycol
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100.
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 5-5000 µg/plate b) Without metabolic activation: 5-5000 µg/plate
Vehicle	water
Remarks - Method	No tests were conducted using *E. coli* strains. Therefore, due to cytotoxicity at higher concentrations in the range-finding test, the test was repeated at lower concentrations in the main study. No other significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 500	≥ 500	> 5000	Negative
Test 2		500	> 500	Negative
Present				
Test 1	5000	≥ 500	> 5000	Negative
Test 2		500	> 500	Negative

Remarks - Results	Cytotoxicity was observed in all *Salmonella* strains at concentrations of 500 µg/plate and greater in the absence and presence of metabolic activation (except for strain TA1535 in the presence of metabolic activation in Test 2). No substantial increases in the number of revertant colonies were seen in any strain either in the presence or absence of metabolic activation. The negative controls were within normal limits and the positive controls (*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine, 9-aminoacridine, 2-nitrofluorene (-S9); 2-aminoanthracene (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Huntingdon Research (1995a)

B.23. Genotoxicity – in vitro

TEST SUBSTANCE	20.3% LAE (notified chemical) 73.5% Propylene glycol

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Cell Type/Cell Line Mouse lymphoma L5178Y cells
Metabolic Activation System Aroclor 1254-induced rat liver S9 fraction
Vehicle Water
Remarks - Method No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	100*, 150, 200*, 220, 240, 260, 280*, 300*	3 hours	48 hours	11-12 days
Test 2	100, 150*, 200, 220*, 240*, 260, 280*, 300	3 hours	48 hours	11-12 days
Present				
Test 1	100, 200*, 300*, 375, 400, 425*, 450*, 500	3 hours	48 hours	11-12 days
Test 2	100, 200*, 300*, 375, 400*, 425, 450*, 500	3 hours	48 hours	11-12 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 250	≥ 280	> 300	Negative
Test 2	-	≥ 240	> 300	Negative
Present				
Test 1	≥ 500	≥ 400	> 500	Negative
Test 2	-	≥ 425	> 500	Negative

Remarks - Results The negative controls were within normal limits and the positive controls (Ethyl methanesulfonate (-S9); 20-Methylcholanthrene (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

No significant increase in the frequency of mutant cells was observed.

CONCLUSION The notified chemical was not mutagenic to L5178Y mouse lymphoma cells treated *in vitro* under the conditions of the test.

TEST FACILITY Huntingdon Research (1995b)

B.24. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (88.2%)

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain L5178Y Mice
Cell Type/Cell Line Lymphoma
Metabolic Activation System Aroclor 1254-induced rat liver S9 fraction
Vehicle DMSO
Remarks - Method No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10*, 24*, 28*, 30*, 34, 38, 40, 45, 50	3 hours	24 and 48 hours
Test 2	10*, 24*, 26*, 28*, 30*, 31*, 32*, 33*, 34*	3 hours	24 and 48 hours
Test 3	1*, 10*, 20*, 32*, 34*, 38*, 40*, 45*	24 hours	24 and 48 hours
Test 4	1*, 20*, 30*, 40*, 42.5*, 45*, 47.5*, 50*	24 hours	24 and 48 hours

Present			
Test 1	10*, 40*, 42*, 43*, 45, 46*, 47, 48, 50	3 hours	24 and 48 hours
Test 2	15*, 30*, 42*, 43*, 43.5*, 44, 44.5*, 45*, 45.5*, 46*	3 hours	24 and 48 hours
Test 3	-	-	-
Test 4	-	24 hours	24 and 48 hours

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 30	≥ 600	Negative
Test 2	≥ 30	-	Negative
Test 3	≥ 30	≥ 38	Negative
Test 4	≥ 30	≥ 42.5	Negative
Present			
Test 1	≥ 40	-	Negative
Test 2	≥ 43.5	-	Negative
Test 3	-	-	-
Test 4	-	-	-

Remarks - Results

The negative controls were within normal limits and the positive controls (Methyl methanesulfonate (-S9); 3-methylcholanthrene (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

No significant increase in the percentage of cells with chromosomal aberrations was observed in the absence or presence of metabolic activation in any of the treatments.

CONCLUSION

The notified chemical was not clastogenic to L5178Y Mice Lymphoma cells treated *in vitro* under the conditions of the test.

TEST FACILITY

Huntingdon Life Sciences (2004)

B.25. Genotoxicity – in vitro

TEST SUBSTANCE

20.3% LAE (notified chemical)
73.5% Propylene glycol

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line: Cultured human lymphocytes
Metabolic Activation System: Aroclor 1254-induced rat liver S9 fraction
Vehicle: water
Remarks - Method: No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000	3 hours	18 hours
Test 2	10, 125, 250, 500, 750, 1000, 1500, 2000	3 hours	18 hours
Test 2	10, 125, 250, 500, 1000, 2000	3 hours	32 hours
Present			
Test 1	10, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000	3 hours	18 hours
Test 2	10, 125, 500, 600, 700, 800, 1000	3 hours	18 hours
Test 2	10, 250, 500, 1000	3 hours	32 hours

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 1000	2000	Negative
Test 2	≥ 1000	≥ 1000	Negative
Test 2	≥ 1000	-	Negative
Present			
Test 1	≥ 1000	≥ 250	Negative
Test 2	> 1000	≥ 700	Negative
Test 2	> 1000	-	Negative

Remarks - Results

The negative controls were within normal limits and the positive controls (Ethyl methanesulfonate (-S9); Cyclophosphamide (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

No significant increase in the percentage of cells with chromosomal aberrations was observed in the absence or presence of metabolic activation in any of the type of treatments. Precipitation was observed at ≥ 1000 in the absence of metabolic activation and at ≥ 250 in the presence of metabolic activation.

In Test 1, the mitotic index was reduced to 7% in the absence of metabolic activation and 18% in the presence of metabolic activation at a concentration of 1000 μg/mL. Similarly, in Test 2, at a concentration of 1000 μg/mL the mitotic index was reduced to 32% in the absence of metabolic activation and to 61% in the presence of metabolic activation at the 18 hour harvest. At the 32 hour harvest, the mitotic index was reduced to 17% in the absence of metabolic activation and no significant reduction in the mitotic index was observed in the presence of metabolic activation.

CONCLUSION

The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY

Huntingdon Research (1995c)

B.26. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical (89.4%)

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line: Human blood lymphocytes

Metabolic Activation System: Aroclor 1254-induced rat liver S9 fraction

Vehicle: DMSO

Remarks - Method: No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	12.5, 25, 50*, 100*, 200*, 400, 800, 1600	3 hours	20 hours
Test 2	12.5, 25, 50*, 100*, 200*, 400, 800, 1600	3 hours	20 hours
Present			
Test 1	12.5, 25, 50*, 75, 100*, 150*, 200*, 300	20 hours	20 hours
Test 2	25, 50*, 100*, 150*, 200, 300	3 hours	20 hours

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 200	≥ 400	Polyploidy
Test 2	≥ 150	-	Polyploidy
Present			
Test 1	≥ 200	≥ 400	Polyploidy
Test 2	≥ 200	-	Polyploidy

Remarks - Results

The negative controls were within normal limits and the positive controls (Mitomycin C (-S9); Cyclophosphamide (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

No significant increase in the percentage of cells with chromosomal aberrations was observed in the absence or presence of metabolic activation. Precipitation was observed at ≥ 400 in Test 1.
In Test 1, the mitotic index was reduced to 32% in the absence of metabolic activation and 31% in the presence of metabolic activation at a concentration of 200 μg/mL. Similarly, in Test 2, at a concentration of 150 μg/mL the mitotic index was reduced to 32% in the absence of metabolic activation and 57% in the presence of metabolic activation. A corresponding statistically significant increase ($P < 0.01$) in polyploidy was observed at 200 μg/mL in Test 1 and at 100 and 150 μg/mL in Test 2 with and without metabolic activation. An increased incidence of polyploid cells may give an indication of the potential of a chemical to induce aneuploidy. However, polyploidy alone does not indicate aneugenic potential and may simply indicate cell cycle perturbation; it is also commonly associated with increased cytotoxicity. However, in the absence of further investigations regarding the chemicals potential to induce aneuploidy, the notified chemical may be considered to be a concern for mutagenicity.

CONCLUSION

The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test. However, there was a significant reduction in the mitotic index and polyploidy was observed at 100, 150 and 200 μg/mL. The study authors consider these findings to be related to toxicity and not biologically relevant because incidence of polyploidy was observed at cytotoxic concentrations. However, it indicates that the notified chemical perturbs the normal cell cycle and it may also indicate the potential of the notified chemical to induce aneuploidy.

TEST FACILITY

Huntingdon Life Sciences Ltd (2001e)

B.27. In vitro stability

TEST SUBSTANCE

Carbon-14 radiolabelled notified chemical (labelled on the arginine carbons)
Specific activity: 10000 dpm/μg
Chemical purity: 95.62%
Radiochemical purity: > 95%

METHOD

The study investigated the stability of the notified chemical (LAE) in simulated gastric and intestinal fluids (at pH 6.8 and 7.5), and in human plasma and a preparation of human hepatocytes.

Gastric and intestinal fluids

Concentration of LAE added: ~0.25mg/ml
Simulated gastric fluid with or without pepsin: Sodium chloride was dissolved in dilute hydrochloric acid, with or without pepsin, as appropriate. The pH was measured to be 0.91 and 0.95, respectively.
Simulated intestinal fluid with or without pancreatin (pH 6.8 and 7.5): Monobasic potassium phosphate was dissolved in water with sodium hydroxide added to adjust the pH or 6.8 or 7.5, then pancreatin was added, as appropriate.

Plasma and hepatocytes
Concentration of LAE added: ~10µg/ml
Plasma: blood was taken from four volunteers and the plasma separated.
Hepatocytes: male human cryopreserved hepatocytes were thawed and pooled together prior to use.

Incubation was performed at 37°C and samples taken at intervals for up to 4 hours. Samples were analysed by HPLC.

Remarks - Method GLP compliant. In-house procedures were followed.

RESULTS

Remarks - Results

Simulated gastric fluid
The notified chemical was stable in simulated gastric fluid, with and without pepsin, over a two-hour period. Small amounts of **N^{α}-Lauroyl-L-Arginine (LAS)** were present in some samples.

Simulated intestinal fluid
In simulated intestinal fluids containing pancreatin (at both pH 6.8 and pH 7.5), LAE was quickly degraded to LAS and then to arginine. At the zero time point, LAS and arginine represented > 95% and < 5% sample radioactivity, respectively. After 4 hours the proportions were reversed, with the respective radioactivities being < 6% and > 94%. LAE was not detected in any of the samples.

In the absence of pancreatin the notified chemical was more stable. At pH 7.5, the notified chemical represented at least 99.6% of the sample radioactivity in all samples up to the 4 hour time point. At pH 6.8, LAE was stable for 30 minutes (at least 98.1% radioactivity). It then began to degrade to LAS, and after 4 hours, the notified chemical represented 80.6% sample radioactivity and LAS the balance, 19.4%.

Human plasma
In human plasma LAS was the only degradation product of the notified chemical, representing an average of 46.7% sample radioactivity after 4 hours. Arginine was not detected in these samples.

Human hepatocytes
The notified chemical was degraded in the presence of human hepatocytes. At the zero time point, the notified chemical represented 75.7% sample radioactivity and LAS 24.4%. After 3 hours this had changed to 6.1% and 81.2%, respectively. In the absence of hepatocytes the notified chemical was similarly degraded to LAS. After 3 hours the notified chemical declined to 12.5% while LAS represented 84.2%. Arginine was not detected in any of these samples.

Hydrolysis was enzyme-mediated: in simulated intestinal fluids with pancreatin at both pH 6.8 and 7.5. Without pancreatin, LAE was stable at pH 7.5 (over 4 hours), while degradation to LAS was considerably slowed at pH 6.8.

LAE was degraded to LAS (but not arginine) by human plasma and human hepatocytes over 4 and 3 hours, respectively.

CONCLUSION	The notified chemical was stable in simulated gastric fluid for at least 2 hours. In simulated intestinal fluid, hydrolysis was enzyme mediated, as it occurred much more rapidly in the presence of pancreatin. The notified chemical was hydrolysed to LAS by human plasma and hepatocytes.
TEST FACILITY	Huntingdon Life Sciences Ltd (2003a)

B.28. Metabolism in the rat

TEST SUBSTANCE	Carbon-14 radiolabelled notified chemical (LAE) (labelled on the arginine carbons) Chemical purity: not stated Radiochemical purity: 99.4%

METHOD

Species/Strain	Rat, Sprague Dawley Crl:CD BR, 4 males
Route of Administration	Oral – gastric intubation
Vehicle	1% aqueous methyl cellulose
Dose	177 - 180 mg radiolabelled LAE/kg bw
Sample Collection	Urine and expired air were collected from each animal at 0-8, 8-24hr and then at 24 hour intervals. Faeces were collected from each animal at 24 hr intervals.
Sacrifice	120 hours after dose administration
Remarks - Method	GLP compliant. In-house procedures were followed

RESULTS

In the 5 days after dosing a mean of 36.6% of the radioactivity of the dose was excreted as CO_2 in expired air, 11.8% in urine and 4.3% in faeces. A mean of 46.4% of the radioactivity of the dose remained in the carcass at sacrifice with a mean of 3.4% in the liver and 2.0% in the gastrointestinal tract. The mean recovery of radioactivity was 99.5%. Analysis of urine showed that the major radioactive component co-chromatographed with urea, though the identity of the metabolite was not confirmed. Urea in urine represented a mean of 7.7% of the radioactivity of the dose administered.

CONCLUSION

The test substance is likely to have been well absorbed and rapidly metabolised. The authors propose that this suggests that the notified chemical is rapidly metabolised by hydrolysis to arginine where it subsequently undergoes natural amino acid catabolism and is ultimately eliminated as carbon dioxide and urea in urine. This is consistent with the high retention of radioactivity in the carcass and liver of the rats.

TEST FACILITY	Huntingdon Life Sciences Ltd (1998a)

B.29. In vivo and in vitro metabolism in the rat

TEST SUBSTANCE	Carbon-14 radiolabelled notified chemical (LAE) (labelled on the arginine carbons) Chemical purity: 89.4% Radiochemical purity: 99.8%

METHOD

Species/Strain	Rat, Sprague Dawley Crl:CD BR
Route of Administration	Oral – gastric intubation
Vehicle	1% aqueous methyl cellulose
Dose	200 mg radiolabelled LAE/kg bw *In vitro*: 10 µg ^{14}C-LAE/mL (8.9 µg ethyl lauroyl arginate HCl) *In vivo*: 6 rats received 200 mg ^{14}C-LAE/kg bw (178.8 mg ethyl lauroyl arginate HCl/kg bw)
Sample Collection	*In vitro* S9 liver fraction: Treated with the test substance, incubated at 37ºC, and samples collected after 4, 6 and 24 h. *In vitro* control plasma: Plasma from control rats was treated with the test

	substance, incubated at 37ºC, and samples taken at 0, 1 and 4 h. *In vivo* blood: Following treatment with the test substance, pairs of rats were anaesthetised and blood samples taken at 0.5, 1 and 4 h after treatment.
Remarks - Method	GLP compliant. In-house procedures were followed.

RESULTS

In vitro:

Incubation of the test substance with S9 liver fractions and plasma showed metabolism of LAE. In S9 samples, unmetabolised LAE (N^{α}-Lauroyl-L-arginine ethyl ester), arginine, ornithine and urea were identified, with ornithine being the major metabolite. Four hours after treatment more than 50% of the notified chemical had been metabolised and by 24 hours approximately 25% was detected. No significant degradation of ^{14}C-LAE occurred in a control incubation sample (containing no S9 fraction).

Incubation of plasma for up to 4 hours showed rapid hydrolysis of Ethyl lauroyl arginate HCl to LAS (N^{α}-Lauroyl-L-arginine) and arginine. Arginine was then further metabolised to ornithine. The plasma extracts showed similar profiles to that observed in S9 fractions. Some bonding of radioactivity to plasma proteins is assumed to occur, given that the extraction of radioactivity decreased over time during this experiment from 99.5% at zero time to 86.4% at 4 hours.

In vivo:

In rats that had been treated with a single oral dose of ^{14}C-LAE, the concentration of total radioactivity in plasma increased over time from a mean of 14.2 µg equivalents LAE/mL plasma 0.5hr after dosing to 118 µg equivalents LAE/mL plasma 4 hours after dosing. Extraction of radioactivity from plasma decreased over time from a mean of 74.8% total radioactive residue (TRR) at 0.5 hours to mean of 19.7% TRR 4 hours after dosing.

LAE and LAS both represented a mean of less than 10% TRR at all sample times. Arginine was the major metabolite present, with a mean maximum of 48.4% TRR 0.5hr after dosing, and then decreasing to a mean of 9.6% TRR after 4 hours. Ornithine was present at a mean maximum of 7.7% TRR after 0.5hr, and then declined to 1.6% after 4 hours. Unidentified polar material was also present at a maximum of 17.4% TRR 1 hour after dosing, and later decreasing.

CONCLUSION

The results of this study suggest that the test substance and/or its metabolites bind to plasma proteins and/or are naturally incorporated (as indicated by the increase in non-extractable radioactivity observed both *in vitro* and *in vivo*). The results also suggest that the test substance is rapidly metabolised by hydrolysis of the ethyl ester and lauroyl amide to arginine, followed by further catabolism to ornithine and urea.

TEST FACILITY	Huntingdon Life Sciences Ltd (2001f)

B.30. Pharmacokinetics in Rats

TEST SUBSTANCE	Ethyl lauroyl arginate HCl (91.87%)
METHOD	
Species/Strain	Rat, Sprague Dawley Crl:CD BR Pilot study: 4 males and 4 females Main study: 4 males/group; total 20
Route of Administration	Oral – Gavage
Dose and vehicle	**Pilot:** Single 40 mg LAE/kg bw dose in propylene glycol/water **Main:** In propylene glycol/water: Single 40, 120 or 320 mg LAE/kg bw dose In glycerol/water or water alone: single 120 mg LAE/kg bw dose. All dose solutions were prepared on the day of dosing and mixed at a ratio of 1:3.5 LAE:solvent and to volume with distilled water.
Sample Collection	**Pilot:** Blood was taken from a tail vein at 15, 30, 60, 90, 120 and 240 minutes

	Main: Blood was taken from a tail vein at 30, 60, 90, 120 and 240 minutes and 8 hours after dosing. Plasma was separated from the blood immediately after sampling and then processed.
Remarks - Method	GLP compliant. In-house procedures were followed.

RESULTS

The results from the pilot experiment were used to determine the appropriate dose levels, sampling regimen and numbers of animals used in the main study. As there were no significant differences observed between males and females, only males were used in the main study.

Main Study

The pharmacokinetic parameters of LAE and its main breakdown product, LAS, in propylene glycol/water are summarised below (standard deviations, where available, are shown in parentheses)**:**

Dose Level (mg/kg bw)	**Cmax (ng/mL)**		**AUC_8 (ng.h/mL)**	
	LAE	**LAS**	**LAE**	**LAS**
40	2.02 (1.28)	24.2 (31.9)	-	52.5 (45.0)
120	1.23 (0.29)	23.2 (2.5)	-	103 (8.0)
320	2.60 (1.81)	96.9 (79.7)	7.50 (1.13)	315 (58)

- Could not be calculated

Where:

C_{max} = maximum plasma concentration

AUC_8 = area under plasma concentration-time curve up to 8 hours post-dose

The time at which the maximum plasma concentration of LAE occurred was generally 0.5 or 1.0 hour post-dose for the 40 and 120 mg/kg bw doses and between 0.5 and 4 hours for 320 mg/kg bw doses indicating that absorption was generally rapid. The maximum plasma concentrations for LAS were similar to that of LAE.

The ratios between Cmax and AUC8 are shown below:

Dose Level (mg/kg bw)	**Dose Level Ratio**	**Cmax Ratio**		**AUC_8 Ratio**	
		LAE	**LAS**	**LAE**	**LAS**
40	1.0	1.0	1.0	-	1.0
120	3.0	0.6	1.0	-	2.0
320	8.0	0.3	4.0	-	6.0

The plasma concentration of LAE (Cmax, rate of systemic exposure) did not appear to increase consistently with increasing dose. The extent of systemic exposure (characterised by AUC_8) could not be estimated for some animals due to the small number of quantifiable samples.

The mean rate of systemic exposure (Cmax) of LAS was similar at the lower 2 doses, but was higher at the highest dose level. This increase appeared to be less than the proportionate dose increment, although the marked inter-animal variation should be noted. The mean extent (AUC_8) of systemic exposure to LAS increased by slightly less than the proportionate dose increment over the dose range 40 to 320 mg/kg bw.

Effect of formulation at dose level of 120 mg/kg bw

The vehicle in which the test substance was administered did not have a significant influence upon the key indicators of LAE and LAS plasma concentrations.

CONCLUSION

Concentrations of LAE were generally low and variable, due to rapid hydrolysis to LAS, most likely in the gastrointestinal tract and by tissue and plasma esterases. Concentrations of LAS provide a better indication of relative systemic exposure and absorption of LAE. Changes in LAE formulation did not affect the

pharmacokinetics of LAE or LAS.

TEST FACILITY Huntingdon Life Sciences Ltd (2005b)

B.31. In vitro percutaneous absorption

TEST SUBSTANCE N-α-Lauroyl-L-arginine ethyl ester monohydrochloride (LAE): 90.3%

METHOD

Species/Strain	Female pig skin, unboiled back: 80 kg animal weight
Vehicle	Propylene glycol/water (30/70 solution)
Formulations	0.39% and 1.96%, in propylene glycol/water 30/70 solution. Dose application: 4.8 μL/cm^2, 7 replicates 0.39% Ethyl Lauroyl arginate HCl corresponds to 18.7 μg/cm^2 active substance. 1.96% Ethyl Lauroyl arginate HCl corresponds to 96.5 μg/cm^2 active substance
Remarks – Method	GLP compliant.

Skin preparation

Subcutaneous fat was removed from the skin, it was rinsed with tap water, and the bristles cut off. It was then dermatomed to a thickness of 700 μm and punched to an appropriate diameter. Intact skin discs were prepared to fit the diffusion cells. The integrity of skin membranes was checked by measuring Transepidermal Water Loss. The diffusion cells were stabilised for one hour in a water bath maintained at 32 ºC (normal skin temperature).

Application

The test formulation (9 μL) was applied to the epidermal surface (1.86 cm^2 exposed surface; 4.8 μL /cm^2 skin) and kept in contact with the skin for 24 hours.

Sampling

At the end of the 24 hr contact period the receptor fluid was recovered.
The skin bottom and lower section of the diffusion cell were washed with distilled water for recovery.
Test solution remaining on the skin surface was washed off with water and collected.
Eight skin strippings were carried out uniformly on the stratum corneum in order to remove it.
The epidermis was separated from the dermis by heating the skin at 80 ºC for a few seconds.

RESULTS

Following application of the 0.39% formulation, the quantities of active ingredient were below the limit of quantification (ie. below 4.84 mg/L) in all compartments analysed.

Following application of the 1.96% formulation, the quantities of active ingredient found in the compartments analysed were as follows:

Compartment	Quantity (μg/cm^2)	% of applied dose
Surface	56.09±10.79	59.05±11.36
Stratum corneum	28.80±9.04	30.33±9.51
Epidermis	3.78±1.84	3.98±1.94
Dermis	1.46±1.65	1.54±1.74
Receptor fluid	not detected	not detected

Under the conditions of this study, the percutaneous absorption of 1.96% LAE HCl in propylene glycol/water

(30/70)) after an exposure time of 24 hours was found to be 5.24 ± 2.29 μg/cm^2. This corresponds to a mean total absorption of 5.52% into the epidermis and dermis.

CONCLUSION	The notified chemical was found to absorb into pig skin.
TEST FACILITY	Instituto de Investigaciones Quimicas Y Ambientales de Barcelona (2002)

B.32. Preliminary determination of breakdown products in plasma after oral administration to healthy male volunteers

TEST SUBSTANCE	Carbon-13 radiolabelled notified chemical (LAE) (labelled on the arginine carbons) Chemical purity: 86.7% Radiochemical purity: 96%
METHOD	Dose: 5 mg/kg bodyweight Vehicle: 15 mg/kg bodyweight propylene glycol made up to 1 ml/kg bodyweight with purified water Two healthy male volunteers received an oral solution of the test substance. Blood samples were taken pre-dose, and at 5, 10, 15, 30 min, 1, 2, 4, 8, 12, and 24 hours post-dose. The resulting plasma samples were stored frozen and a total of 22 samples were analysed. Plasma concentrations of ^{13}C-LAE, ^{13}C-LAS and ^{13}C-arginine in the samples were determined using liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method.
Remarks - Method	GLP compliant. In-house procedures were followed.
RESULTS	
Remarks - Results	Plasma levels of ^{13}C-LAE and ^{13}C-LAS ranged from below the limit of quantification (1 ng/mL) to 44.0 ng/mL and 154 ng/mL, respectively, while those of ^{13}C-arginine ranged from below the limit of quantification (10 ng/mL) to 680 ng/mL. *Clinical results* Both male subjects reported a burning sensation in the throat, whilst one also reported experiencing nausea. The study authors assumed that the burning sensation, and possibly the nausea, was due to the solvent, propylene glycol (15 mg/kg bw). No support was provided for the statement. It is noted that the WHO acceptable daily intake of propylene glycol is up to 25 mg/kg.
CONCLUSION	The notified chemical appeared to be well-tolerated by the male volunteers, with the exception of the burning sensation and nausea noted above. LAE appears to degrade to LAS and arginine in humans, as indicated by levels in human plasma.
TEST FACILITY	CentraLabS (2005a)

B.33. Determination of breakdown products in plasma after oral administration to healthy male volunteers

TEST SUBSTANCE	Carbon-13 radiolabelled notified chemical (LAE) (labelled on the arginine carbons) Chemical purity: 86.7% Radiochemical purity: 96%
METHOD	Dose and vehicle:

2 subjects received 2.5 mg/kg bodyweight test substance with 7.5 mg/kg bw propylene glycol (made up to 1 ml/kg bodyweight with purified water).
4 subjects received 1.5 mg/kg bodyweight test substance with 4.5 mg/kg bw propylene glycol (made up to 1 ml/kg bodyweight with purified water).

Subjects:
Six healthy male volunteers, mean age 33.7 years, mean weight 79.77 kg, mean body mass index 24.87 kg/m^2.

Subjects were administered the test substance orally. Blood samples were taken pre-dose, and at 5, 10, 15, 30 min, 1, 2, 4, 8, 12, and 24 hours post-dose. The resulting plasma samples were stored frozen in tubes containing a stabilising agent (sodium metabisulphite) and a total of 66 duplicate samples were analysed.

Plasma concentrations of ^{13}C-LAE, ^{13}C-LAS and ^{13}C-arginine in the samples were determined using liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method.

Remarks - Method GLP compliant. In-house procedures were followed.

RESULTS

Remarks - Results Plasma concentrations of ^{13}C-LAE were below the limit of quantification at all sampling times and in all subjects, with the exception of one, who displayed quantifiable concentrations at 10 and 15 minutes post dose (subject had been treated with 2.5 mg/kg bw). This meant that the pharmacokinetics of ^{13}C-LAE could not be meaningfully assessed.

The pharmacokinetic parameters of the breakdown products of ^{13}C-LAE (^{13}C-LAS and ^{13}C-arginine) are summarised below: (standard deviations, where available, are shown in parentheses).

^{13}C-LAS	**Dose mg/kg**	**Cmax ng/mL**	**Tmax hrs**	**AUC_t ng.h/mL**	**AUC ng.h/mL**	**λz $hours^{-1}$**	**$t_{1/2}$ hrs**
	1.5	18.2 (8.6)	2 [a]	90.6 (35.3)	96.4 (34.3)	0.2806 (0.0464)	2.5[b]
	2.5	23.9	1.5 [a]	118	128	0.2866	2.4[b]
^{13}C-Arginine	**Dose mg/kg**	**Cmax ng/mL**	**Tmax hrs**	**AUC_t ng.h/mL**	**AUC ng.h/mL**	**λz $hours^{-1}$**	**$t_{1/2}$ hrs**
	1.5	124 (12)	0.75[a]	383 (103)	556 (142)	0.2891 (0.1261)	2.4[b]
	2.5	240	1.25 [a]	764	864	0.2862	2.4[b]

a – median b – calculated as ln2/mean λ_z

Where:
AUC = area under plasma concentration-time curve extrapolated to infinite time
AUC_t = area under plasma concentration-time curve up to the time of the last quantifiable sample
C_{max} = maximum plasma concentration
λ_z = terminal rate constant
$t_{1/2}$ = terminal half-life
T_{max} = Time at which C_{max} occurred

Tmax for ^{13}C-arginine occurred either earlier or at the same time as Tmax for ^{13}C-LAS, indicating that the absorption of ^{13}C-arginine from the gastrointestinal tract to the blood occurred more rapidly than ^{13}C-LAS (from which it was broken down).
Plasma concentrations of ^{13}C-arginine tended to be considerably higher than

those of ^{13}C-LAS (this difference would be even greater if the approximate two-fold difference in molecular weight was accounted for). This indicates that there was relatively extensive breakdown of LAE to arginine.
The terminal half-life (t ½) of ^{13}C-LAS was in the range 2.2 to 3.3 hours, and appeared similar to that of ^{13}C-arginine (1.6 to 4.0 hours). However, these results should be interpreted with caution, as the t ½ values of arginine could not be acceptably determined for some of the subjects.

Clinical results
Mild adverse effects were reported by 2 subjects at each dose level. Following the 2.5 mg/kg bw dose, one subject experienced headaches, and following the 1.5 mg/kg bw dose, one subject experienced diarrhoea and flatulence, which occurred approximately 30 hours after dosing. The study authors considered that these effects were not likely to be related to treatment as similar effects were not observed at the other dose levels in this study or in the preliminary study. In addition, the authors state that preclinical studies using the test substance have not resulted in effects of this sort.

CONCLUSION — In conclusion, there was insufficient quantifiable data to allow meaningful assessment of the pharmacokinetics of ^{13}C-LAE, but it was possible to determine the Cmax, AUC_t, AUC, terminal rate constant and terminal half-life of ^{13}C-LAS and ^{13}C-arginine, its breakdown products. LAE appears to break down to LAS and arginine in humans, as indicated by levels in human plasma.

TEST FACILITY — CentraLabS (2005b)

B.34. Toxicity to reproduction – preliminary range finding study (1)

TEST SUBSTANCE — Notified chemical (LAE) (69.1%)
METHOD — This preliminary study does not follow an official guideline. The study was conducted in compliance with GLP.
Species/Strain — Rabbit/New Zealand White
Route of administration — Oral gavage
Vehicle — 1% w/v aqueous methylcellulose
Remarks - Method — *Staircase phase*: Two non-pregnant female rabbits were dosed for 10 days starting from 41.5 mg/kg/day LAE (after adjusting for test material purity), with the dosage approximately doubling every two days until reaching a dosage maximum of 691 mg/kg/day of LAE. Animals were sacrificed on Day 11.
Constant dosage phase: Two female rabbits were naturally mated with New Zealand white males and females were injected intravenously with 25 i.u. luteinizing hormone to ensure successful ovulation and conception. The day of mating was designated Day 0 of gestation. The pregnant females were then given a dose of 691 mg/kg/day for seven consecutive days from Day 6 to Day 12 of gestation. Animals were terminated on Day 13 of gestation.

Non-pregnant females

	Day of dosing									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Animal Number	*Dose of notified chemical (mg/kg/day)*									
1	41.5	41.5	82.9	82.9	172.8	172.8	346.0	346.0	691.0	691.0
2	41.5	41.5	82.9	82.9	172.8	172.8	346.0	346.0	691.0	691.0

Pregnant females

	Day of gestation												
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Animal Number	*Dose of notified chemical (mg/kg/day)*												
3	0	0	0	0	0	0	691.0	691.0	691.0	691.0	691.0	691.0	691.0
4	0	0	0	0	0	0	691.0	691.0	691.0	691.0	691.0	691.0	691.0

RESULTS

Remarks - Results	*Staircase phase*: No deaths occurred and there was no change in the general condition of the females during the study. Bodyweight gain was not significantly affected by treatment although a marginal loss of weight was recorded in Animal No. 1 on Day 10 and in Animal No. 2 on Day 9 of dosing. There were no adverse findings at necropsy. Animal No. 2 showed yellow staining on forepaws, hindpaws and tail from Day 1 to the end of the study period. At necropsy, Animal No. 2 had brown stained fur on tail, hindlimbs and around the urinogenital area. *Constant dosage phase*: No deaths occurred. Both females showed reduced water intake on Gestation Days 8-9 and noticeable reduction in food intake from Day 8 to the end of the study period. This was accompanied by reduction in bodyweight gain and reduced faecal production. Animal No. 4 became stressed during dosing on Gestation Day 7 that lead to a delay in dosing by 30 minutes. On Gestation Day 8, Animal No. 4 became underactive, had irregular respiration, blue extremities and hunched posture 1 hour after dosing and remained underactive with irregular respiration for around 5 hours afterwards. Noisy respiration was observed for the remainder of the treatment period until termination. At necropsy, Animal No. 3 had collapsed areas of the lungs, which appeared slightly dark in colour. Animal No. 4 showed collapsed lungs and evidence of pale raised areas on the lung surface and these findings suggested that lung infection had occurred. Wet and yellow-stained fur around the urino-genital region and on all limbs was noted in the same animal. The kidneys of both animals had numerous prominent dark blood vessels on the surface. Both females were pregnant at termination and there were no apparent adverse findings on the embryo.
CONCLUSION	The complications in the lungs of the constant dosage pregnant females were not regarded as treatment-related changes and treatment at 691 mg/kg/day of LAE (equates to administered dose of 1000 mg/kg/day) did not result in significant effects on embryo survival. Hence the highest dosage for the preliminary teratology study should be 1000 mg/kg/day.
TEST FACILITY	Huntingdon Life Sciences Ltd (1998b)

B.35. Toxicity to reproduction – preliminary range finding study (2)

TEST SUBSTANCE	Notified chemical (LAE) (69.1%)
METHOD	This preliminary study does not follow an official guideline. The study was conducted in compliance with GLP.
Species/Strain	Rat/Charles River Crl:CD BR
Route of administration	Oral gavage
Vehicle	1% w/v aqueous methylcellulose
Remarks - Method	*Staircase phase*: Four non-pregnant female rats were dosed for 8 days starting from 172.8 mg/kg/day LAE (after adjusting for test material purity), with the dosage approximately doubling every two days until reaching a dosage maximum of 1382 mg/kg/day of LAE. Animals were sacrificed on Day 9 and examined by necropsy. *Constant dosage phase*: Four female rats were naturally mated with males of the same strain. The day on which a sperm positive vaginal smear or at least three copulatory plugs were found was designated Day 0 of gestation. The pregnant females were then given a dose of 1382 mg/kg/day for seven consecutive days from Day 6 to Day 12 of gestation. Animals were terminated on Day 13 of gestation and examined by necropsy.

Non-pregnant females

Day of dosing							
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*

Animal Number	*Dose of notified chemical (mg/kg/day)*							
1	172.8	172.8	346.0	346.0	691.0	691.0	1382.0	1382.0
2	172.8	172.8	346.0	346.0	691.0	691.0	1382.0	1382.0
3	172.8	172.8	346.0	346.0	691.0	691.0	1382.0	1382.0
4	172.8	172.8	346.0	346.0	691.0	691.0	1382.0	1382.0

Pregnant females

	Day of gestation												
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Animal Number	*Dose of notified chemical (mg/kg/day)*												
5	0	0	0	0	0	0	1382	1382	1382	1382	1382	1382	1382
6	0	0	0	0	0	0	1382	1382	1382	1382	1382	1382	1382
7	0	0	0	0	0	0	1382	1382	1382	1382	1382	1382	1382
8	0	0	0	0	0	0	1382	1382	1382	1382	1382	1382	1382

RESULTS

Remarks - Results

Staircase phase: No deaths occurred and there was no change in the general condition of the females during the study. Salivation was observed on a number of occasions immediately after dosing and the frequency of salivation was increased in all animals after Day 4 of dosing at 691 and 1382 mg/kg/day. Bodyweight gain was not significantly affected by treatment although a marginal loss of weight was recorded on Day 8 in three of four animals at the highest dose of 1382 mg/kg/day. Weight gain was recovered on the following day. No toxicologically significant findings were recorded at necropsy.
Constant dosage phase: No deaths occurred and there was no change in the general condition of the females during the study. Occasional salivation was recorded for all animals from Day 10 – 12 of gestation for a short period immediately after dosing. All females were pregnant with 15-17 implantations at termination and the uterus showed one resorbing embryo in three of four females. No adverse findings were recorded at necropsy on maternal organs or embryo survival.

CONCLUSION

The highest dosage for use in a preliminary embryo-foetal study in the rat was determined to be 1382 mg/kg/day of LAE (equates to administered dose of 2000 mg/kg/day).

TEST FACILITY

Huntingdon Life Sciences Ltd (1998c)

B.36. Toxicity to reproduction – preliminary one generation study

TEST SUBSTANCE

Notified chemical (88.2%)

METHOD

Similar to OECD 415 One-Generation Reproduction Toxicity Study

Species/Strain

Crl:CD (SD) IGS BR

Route of Administration

Oral –diet

Exposure Information

Exposure period – **females** (parent – P): 4 weeks before pairing, throughout pairing, gestation, lactation and until termination (after weaning).
F1: same as P, ie. from the time of weaning until termination

Exposure period – **males** (parent – P): 4 weeks before pairing, throughout pairing and until termination
F1: same as P, ie. from the time of weaning until termination

Dose regimen: 7 days per week (continuous)

Vehicle

LAE was mixed in with a standard powdered diet to the desired concentrations (prepared fortnightly).

Remarks - Method

Dosing of parent animals was performed for 4 weeks prior to pairing, rather than the recommended 10 weeks.

Generation	*Group*	*Number and Sex of Animals*	*Dose/Concentration*			*Mortality*
			Nominal	*Actual*	*Notified chemical*	
			M/F ppm	*M/F mg/kg/day*	*M/F mg/kg/day*	
P	1	8/sex	0	0	0	0
Before	2	8/sex	1500	113/123	99.7/108	0
pairing	3	8/sex	5000	380/432	335/381	0
	4	8/sex	15,000	1151/1295	1015/1142	0
F1	1	12/sex	0	0	0	0
3 weeks after	2	12/sex	1500	173/169	153/149	0
selection	3	12/sex	5000	589/586	519/517	0
(averaged)	4	12/sex	15,000	1750/1734	1544/1529	0

RESULTS

Mortality and Time to Death

No unscheduled deaths occurred.

Effects on Parental (P) animals:

Litter survival

At the high dose level, two of the eight litters lost bodyweight between days 1 and 4 of age and were terminated on day 4. At this dose level, there was also a small reduction in survival indices of litters. As such, a possible connection between treatment and slight increases in postnatal litter loss could not be excluded.

Macropathology

In the two females that were terminated after their litters died, mammary tissue appeared inactive or only had small amounts of milk present.

There were no other findings considered to be of toxicological significance.

Effects on 1st Filial Generation (F1)

Sexual maturation

A delay of 4 days in vaginal opening was recorded at the high dose treatment level. The bodyweight of these offspring were also higher than control animals, though there was no consistent relationship between bodyweight and the time of vaginal opening. Balano-preputial separation in males was unaffected by treatment.

Oestrous cycles

The first recorded oestrous cycle in treated animals was of 5 days duration for a number of treated animals, as compared to 4 days for control animals. However, the subsequent cycle length was reduced to 4 days in nearly all cases in all groups. It was considered that the normal oestrous cycle was established and that the delay in vaginal opening did not have a lasting impact upon the normal sexual development of the animals.

There were no other findings considered to be of toxicological significance.

Remarks – Results

None

CONCLUSION

Based on the above results, it was concluded that the highest treatment concentration of 15000 ppm was appropriate to be used in the two-generation reproductive toxicity study.

TEST FACILITY Huntingdon Life Sciences Ltd (2003b)

B.37. Toxicity to reproduction – two generation study

TEST SUBSTANCE	Notified chemical (88.2%)
METHOD	OECD 416 Two-Generation Reproduction Toxicity Study
Species/Strain	Crl:CD (SD) IGS BR
Route of Administration	Oral –diet
Exposure Information	Exposure period – **females** (parent – P): 10 weeks before pairing, throughout pairing, gestation, lactation and until termination. F1: same as P until termination Exposure period – **males** (parent – P): 10 weeks before pairing, throughout pairing and until termination F1: same as P until termination Dose regimen: 7 days per week (continuous)
Vehicle	LAE was mixed in with a standard powdered diet to the desired concentrations (prepared fortnightly).
Remarks - Method	No significant protocol deviations.

Weeks on study	*P*	*F1*	*F2*
1-11	Exposure of P animals prior to first mating		
11-13	P mating period/gestation		
14-16	P lactation	F1 born and litter size adjusted to 10 offspring	
17-20	Exposure of P animals ceased	F1 weaned; treatment of F1 animals begins	
	P males and females killed	F1 unselected offspring killed	
27-29		F1 mating period/gestation	
30-32		F1 lactation	F2 born and litter size adjusted to 10 offspring
33-35		Exposure of P animals ceased and animals killed	F2 offspring killed

Generation	*Group*	*Number and Sex of Animals*	*Dose/Concentration*		
			Nominal	*Actual*	*Notified chemical*
			Male/Female ppm	*Male/Female mg/kg/day*	*Male/Female mg/kg/day*
P	1	28/sex	0	0	0
Before pairing	2	28/sex	2500	181/207	160/183
	3	28/sex	6000	434/502	383/443
	4	28/sex	15,000	1073/1226	946/1081
P	1	28/sex	0	0	0
During gestation	2	28/sex	2500	-/231	-/204
	3	28/sex	6000	-/585	-/516
	4	28/sex	15,000	-/1518	-/1339
P	1	28/sex	0	0	0
During lactation	2	28/sex	2500	-/402	-/355
	3	28/sex	6000	-/1018	-/898
	4	28/sex	15,000	-/2600	-/2293
F1	1	24/sex	0	0	0
Before pairing	2	24/sex	2500	224/246	198/217
	3	24/sex	6000	537/582	474/513
	4	24/sex	15,000	1356/1489	1196/1313
F1	1	24/sex	0	0	0

During gestation	2	24/sex	2500	-/215	-/190
	3	24/sex	6000	-/535	-/472
	4	24/sex	15,000	-/1430	-/1261
F1	1	28/sex	0	0	0
During lactation	2	28/sex	2500	-/409	-/361
	3	28/sex	6000	-/898	-/792
	4	28/sex	15,000	-/2353	-/2075
F2	1	5/sex	-	-	-
	2	5/sex	-	-	-
	3	5/sex	-	-	-
	4	5/sex	-	-	-

RESULTS

Mortality and Time to Death

Two parental animals were killed for welfare reasons (one low dose male and one high dose female) whilst another was found dead (high dose male) and found to have a malignant nephroblastoma. These deaths were not considered to be treatment-related.

One male in the high dose group in the F1 generation was killed for welfare reasons. In addition, one female from the low dose and one from the high dose group were killed for humane reasons after the offspring were weaned. These deaths were not considered to be treatment-related.

Effects on Parental (P) animals:

Bodyweight

Bodyweight gain during gestation for treated females was greater than controls, however, this finding was not considered to be adverse.

Organ weight

In females treated at the highest dose, on day 28 post-partum the bodyweight relative weights for the spleen and ovaries were statistically significantly reduced compared with controls. This was also the case with ovary weights in low dose females. These effects were not considered to be toxicologically significant as there was no dose dependent trend and the relative weight differences were small (within 10% of controls).

There were no other findings considered to be of toxicological significance.

Effects on 1st Filial Generation (F1)

Bodyweight

There were some minor changes in the body weight of these animals, particularly those in the high dose group, although they were not statistically significant changes.

Sexual maturation

A delay of 4 days in vaginal opening was recorded at the high dose treatment level, which coincided with bodyweights that were significantly higher than control animals. Balano-preputial separation in males was unaffected by treatment.

Gestation

Gestation length and index were unaffected by treatment. One female in the high dose group had a longer gestation length than most of the others (23.5 days, compared to the 22 – 23 days of the others). Given the smaller litter size of this animal, this was considered normal.

Organ weight

There were some minimal effects on organ weights, though they were not considered to be conclusive. The reasons for this is that such effects were not observed in both the absolute weight and the relative weight in the same organ and were not dose related.

There were no other findings considered to be of toxicological significance.

Effects on 2nd Filial Generation (F2)

Litter size and offspring survival

The number of implantations, total litter size, live litter size and offspring survival were considered to be unaffected by treatment. One litter from the control group and one from the low and mid dose groups were killed for welfare reasons.

Bodyweight

A small statistically significant reduction in cumulative bodyweight gain was observed in males and females treated with the high dose for up to 21 days of age. However, this was resolved by day 25. There were also effects on the bodyweight gain of animals treated with the mid dose, though the study authors attribute this to one particular litter.

Pre-weaning examinations

There was some poor performance observed in the startle response for mid and high dose animals compared to controls.

Organ weight

Females treated with the high dose displayed statistically significant reductions in absolute spleen weights (though not dose related), however, the differences were not statistically significant when analysed relative to bodyweight. Males also showed some reductions in spleen weight, though this was not of statistical significance.

There were no other findings considered to be of toxicological significance.

Remarks – Results

There were no adverse effects on any of the parameters measured in this study observed on the P, F1 or F2 generations on exposure to the test substance at concentrations up to 15000 ppm in the diet. Minor effects observed were considered by the study authors to be transient and not of toxicological significance. Based on the above results, the No Observed Adverse Effect Level (NOAEL) for reproductive performance was considered by the study authors to be 15000 ppm LAE. This is equivalent to 1073 mg/kg bw/day of LAE (946 mg/kg bw/day of notified chemical) based on the lowest average intake of adult rats before pairing and up to 2600 mg/kg bw/day LAE (2293 mg/kg bw/day notified chemical) for females during lactation. However, given that delays in vaginal opening of F1 females was observed in this study as well as in the preliminary study, these effects cannot be disregarded (even though they were not associated with other functional changes). This suggests that a NOAEL value of 6000 ppm, corresponding to 502 mg/kg bw/day LAE (443 mg/kg bw/day notified chemical) may be more appropriate.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 502 mg/kg bw/day LAE (443 mg/kg bw/day notified chemical).

TEST FACILITY	Huntingdon Life Sciences Ltd (2005c)

B.38. Developmental toxicity

TEST SUBSTANCE	LAE: Notified chemical (69.1%), water (23.1%)
METHOD	
Species/Strain	Rat/Crl:CD® BR (SD)
Route of Administration	Oral – gavage
Exposure Information	Exposure period: days 6 to 19 of gestation Dose regimen: 0, 200, 600 and 2000 mg/kg bw/day LAE (0, 138, 415, 1382 mg notified chemical/kg bw/day) Control animal received vehicle only.
Vehicle	1% w/v aqueous methyl cellulose
Remarks – Method	As this was a preliminary study, proper study guidelines were not followed. However, the study complied with GLP requirements. Animals were killed on Day 20 of gestation.

RESULTS

Group	*Number of Female Animals*	*LAE (equivalent notified chemical) (mg/kg bw/day)*	*Mortality*
1	6	0 (0)	0
2	6	200 (138)	1 (day 19)
3	6	600 (415)	0
4	6	2000 (1382)	0

Mortality and Time to Death
One female rat in the low dose group (200 mg/kg bw/day) showed reduced food intake, body weight loss (40 g) on days 18-19 of gestation and was killed *in extremis* on gestation day 19. This female rat also had signs of pallor, piloerection, brown staining around one eye, red urine and a perigenital discharge. Necropsy revealed a large amount of dark red fluid within the vagina and both uterine horns. The uterus contained 15 late resorptions.

Effects on Dams
One control animal was considered non-pregnant and was excluded from the study, though staining of uterus revealed a single implantation site.
Salivation after dosing was seen occasionally in the mid dose treatment group (600 mg/kg bw/day) and frequently in the high dose treatment group (2000 mg/kg bw/day) and respiratory noises were also noted for one animal each in the mid and high dose treatment groups. There were no other significant clinical signs recorded in either the control group or any of the treatment groups.
Bodyweight, bodyweight gain, and food consumption were unaffected by treatment.
There were no treatment-related necropsy findings.

Effects on Foetus
There were no treatment related effects on foetal survival or foetal development as assessed by number of live foetuses, foetal weight and macroscopic examination of the foetus at necropsy.

Remarks – Results
One female rat in the low dose group (200 mg/kg bw/day) was killed *in extremis* on gestation day 19. However, in the absence of similar findings at higher dosage groups, it was considered that the findings in this animal were not treatment related. Salivation, seen after dosing particularly in the highest dosage group, is a common non-specific response to gavage treatment, which may relate to the taste or pH of the test material.

CONCLUSION
LAE dosages up to 2000 mg/kg bw had no significant treatment related effects upon food consumption, body weight, and foetal survival or foetal development. A high dose level of 2000 mg/kg bw, considered to be the maximum practical dosage achievable, would be suitable as the highest dose level for the main embryo-foetal study in the rat.

TEST FACILITY Huntingdon Life Sciences Ltd (1998d)

B.39. Developmental toxicity

TEST SUBSTANCE LAE: Notified chemical (69.1%), water (23.1%)

METHOD OECD TG 414
Species/Strain Rat/Crl:CD (SD)
Route of Administration Oral – gavage
Exposure Information Exposure period: days 6 to 19 of gestation
Dose regimen: 0, 200, 600 and 2000 mg/kg bw/day LAE
(0, 138, 415, 1382 mg notified chemical/kg bw/day)
Control animal received vehicle only
Dose volume: 10 ml/kg bw/day
Vehicle 1% w/v aqueous methyl cellulose
Remarks – Method No significant protocol deviations. Dosage levels were chosen based on a

previous dose range-finding study.

RESULTS

Group	*Number of Animals*	*LAE (equivalent notified chemical) (mg/kg bw/day)*	*Mortality*
1	22	0 (0)	0
2	22	200 (138)	0
3	22	600 (415)	2 (day 17 of gestation)
4	22	2000 (1382)	3 (day 7 or 8 of gestation)

Mortality and Time to Death
Three females in the 2000 mg/kg bw/day group were killed *in extremis* on the second and third day of treatment following severe signs of respiratory distress and salivation after dosing. Two of these animals had also shown a significant bodyweight loss prior to sacrifice. Necropsy of all three animals revealed large amounts of gaseous material in the gastro-intestinal tract and implantation sites were grossly normal. In addition, one animal also had enlarged and prominent lymph nodes and another had haemorrhagic lungs, large amounts of pale viscous material in the ileum, reduced and dehydrated caecal contents, dark and enlarged adrenals and a pronounced internal structure of kidneys.

Two animals in the 600 mg/kg bw/day group also showed similar signs of noisy respiration, salivation at the time of dosing and bodyweight losses towards the end of gestation. One of these animals was killed for humane reasons, and the other was killed *in extremis*, both on Day 17 of gestation. Necropsy of these animals revealed large amounts of gaseous material in the gastro-intestinal tract and implantation sites were grossly normal.

Effects on Dams
The general condition of the surviving animals was satisfactory and all the females were pregnant.

Noisy respiration was seen during the treatment period in three animals receiving 200 mg/kg bw/day of test substance, in a total of 7 animals at 600 mg/kg bw/day of test substance, and in 9 animals at 2000 mg/kg bw/day of test substance (including animals which were killed prematurely).

Salivation at the time of dosing was seen in all animals received 2000 mg/kg bw/day of test substance on approximately 50% of dosing occasions, reaching peak daily incidence at about day 14 of gestation. Fourteen animals receiving 600 mg/kg bw/day of test substance occasionally salivated during the dosing period, and at 200 mg/kg bw/day of test substance, salivation was seen once in one animal only.

Neither noisy respiration nor salivation was seen in the control group.

There were no overall treatment related effects upon bodyweight. Occasionally, animals in all treated groups showed transient body weight losses for periods following commencement of treatment on day 6 and some animals receiving 600 mg/kg bw/day of test substance also showed weight loss towards the end of the treatment period. Many of the cases of weight loss coincided with episodes of respiratory distress. There were no similar bodyweight losses in the control group.

There were no overall treatment related effects upon food consumption. However, there were occasional animals in the treatment groups, which showed periods of reduced food intake, which appeared to be associated with episodes of respiratory distress.

There were no maternal necropsy findings at 20 days of gestation considered to be treatment related.

Effects on Foetus
There were no treatment related effects on foetal survival as indicated by the extent of pre- and post-implantation loss and the numbers of live fetuses. Foetal and placental weights and the incidences and types of major foetal abnormalities were unaffected by treatment. The numbers of skeletal and visceral minor abnormalities and variants were also unaffected by treatment.

Remarks – Results
Respiratory distress was recorded among some animals in 600 or 2000 mg/kg bw/day groups. Necropsy of

these animals did not detect damage to the lungs and gross changes were limited to accumulation of gas within the gastrointestinal tract. Therefore, respiratory distress in these animals probably related to aspiration of the increased secretions and/or traces of the irritant dosing material, especially following treatment with the more concentrated/viscous suspensions at the higher doses and is not considered to be a systemic toxic response to oral gavage of the test substance. Although it is difficult to extrapolate respiratory distress observed to man, this may suggest possible bronchial irritation if the test material is inhaled.

Salivation, seen after dosing particularly in the highest dosage group, is a common non-specific response to gavage treatment, which may relate to the taste or pH of the test material and has no toxicological significance.

CONCLUSION
The NOEL for the dam was established as 200 mg/kg bw/day of test substance (equivalent to 138 mg/kg bw/day notified chemical), based on deaths at 600 and 2000 mg/kg bw/day.

The NOEL for the foetus was established as 2000 mg/kg bw/day of test substance (equivalent to 1382 mg/kg bw/day notified chemical), based on no adverse effects observed at the highest dose tested (2000 mg/kg bw/day).

TEST FACILITY Huntingdon Life Sciences Ltd (1998e)

B.40. Developmental toxicity

TEST SUBSTANCE LAE: Notified chemical (69.1%), water (23.1%)

METHOD

Species/Strain	Rabbit/New Zealand White
Route of Administration	Oral – gavage
Exposure Information	Exposure period: days 6 to 19 of gestation Dose regimen: 0, 250, 500 and 1000 mg/kg bw/day LAE (0, 173, 346, 691 mg notified chemical/kg/day) Control animal received vehicle only.
Vehicle	1% w/v aqueous methyl cellulose
Remarks – Method	As this was a preliminary study, proper study guidelines were not followed. However, the study complied with GLP requirements. Animals were killed on Day 29 of gestation.

RESULTS

Group	*Number of Animals*	*LAE (equivalent notified chemical) (mg/kg bw/day)*	*Mortality*
1	6	0 (0)	0
2	4	250 (173)	0
3	4	500 (346)	0
4	4	1000 (691)	0

Mortality and Time to Death
No deaths were recorded.

Effects on Dams
Two animals, one in each of the groups receiving 250 and 1000 mg/kg bw/day, showed periods of respiratory distress during the treatment phase of the study, but this did not appear to be dosage related. There were no other remarkable clinical signs recorded in either the control group or any of the treatment groups.
Reduced food consumption during treatment at 500 and 1000 mg/kg bw/day, leading to slight losses in body weight, were the only effects considered to be treatment related. There were no treatment-related necropsy findings.

Effects on Foetus

There were no treatment related effects on embryo-foetal survival or foetal development as assessed by number of live foetuses, foetal weight and abnormalities seen at necropsy.

Remarks – Results
Both 500 mg/kg bw/day and 1000 mg/kg bw/day doses, were associated with reduced food consumption and reduced body weight gain. No effect was seen on foetuses when the test substance was fed up to the highest dose of 1000 mg/kg bw/day.

CONCLUSION
A dosage of up to 1000 mg/kg bw/day would be suitable as the highest dosage level for the main embryo-foetal study in the rabbit.

TEST FACILITY Huntingdon Life Sciences Ltd (1998f)

B.41. Developmental toxicity

TEST SUBSTANCE LAE: Notified chemical (69.1%), water (23.1%)

METHOD OECD TG 414
Species/Strain Rabbit/New Zealand White
Route of Administration Oral – gavage
Exposure Information Exposure period: days 6 to 19 of gestation
Dose regimen: 0, 100, 300 and 1000 mg/kg bw/day LAE
(0, 69, 207, 691 mg notified chemical/kg/day)
Control animal received vehicle only
Vehicle 1% w/v aqueous methyl cellulose
Remarks – Method No significant protocol deviations. Dosage levels were chosen based on a previous dose range-finding study.
Animals were killed on Day 29 of gestation.

RESULTS

Group	*Number of Animals*	*LAE (equivalent notified chemical) (mg/kg bw/day)*	*Mortality*
1	22	0 (0)	0
2	22	100 (69)	0
3	22	300 (207)	1 (day 14)
4	22	1000 (691)	1 (day 9)

Mortality and Time to Death
At 1000 mg/kg bw/day, one animal was killed on day 9 of gestation following periods of noisy respiration accompanied by reduced food consumption and faecal output, and an aqueous discharge in the cage under tray. Necropsy revealed a small amount of frothy liquid in the trachea, and congestion in the lungs.
At 300 mg/kg bw/day, one animal was also killed on day 14 of gestation because of gasping respiration following dosing. Necropsy revealed incomplete collapse of the lungs, with occasional dark areas of change on the lung surfaces.

Effects on Dams
Reactions to dosing were largely limited to changes in respiration pattern seen in 5 animals each dosed with the test substance at 300 and 1000 mg/kg bw/day (including the two animals, which were killed early in the study and replaced). Adverse respiratory reactions were believed to be associated with a higher risk of irritation being induced during the dosing procedure when high concentrations of test material were used. There were no other signs of adverse reaction to treatment.

One female at 1000 mg/kg bw/day aborted on day 24 of gestation: necropsy revealed three empty implantation sites in the left uterine horn but no implantations in the right horn of the uterus. Two dead foetuses, both of which appeared grossly normal, were found in the under tray of the cage.

Bodyweight gain of animals receiving the test substance at 1000 mg/kg bw/day was slightly but significantly lower than that of the controls throughout most of the treatment period. Bodyweight gain of animals receiving

the test substance at 100 or 300 mg/kg bw/day was similar to that of the controls throughout gestation.

Food consumption by animals receiving the test substance at 1000 mg/kg bw/day fell slightly when treatment started and was significantly lower than that of the control group during days 13-19 of gestation but recovered to similar to control levels after completion of the dosing period. Food consumption at 100 and 300 mg/kg bw/day was unaffected by treatment.

There were no necropsy findings for females killed on day 29 after mating, which were considered to be related to treatment with test substance.

Effects on Foetus
There were no apparent treatment related effects on foetal survival.
The numbers of corpora lutea, implantations and live young in the control group were generally lower than in the treated groups but intergroup differences were not statistically significant. In the control, low, mid and high dose groups respectively, the pre-implantation losses were 8.9%, 13.4%, 12.3% and 11.3%, and the post-implantation losses were 9.3%, 11.9%, 10.3% and 6.9%. Litter sizes (live foetuses) in the controls to high dose groups respectively were 8.9, 9.1, 9.1 and 10.0.

There were no effects of treatment on foetal weight or placental weight. The incidences and types of major foetal abnormalities were unaffected by the treatment. The numbers of minor skeletal and visceral abnormalities and variants were unaffected by the treatment.

Remarks – Results
Treatment of rabbits with the test substance by oral gavage at 300 or 1000 mg/kg bw/day was associated with difficulty in dosing and signs of respiratory distress in some animals. Similar respiratory signs were recorded during the preliminary study and in the rat developmental study assessed in this assessment report. This clinical sign was related to aspiration of traces of the test material and altering the standard dosing procedure, by using a clean moist catheter instead of a clean dry catheter, appeared to alleviate some of the dosing problems. However, there was still a residual incidence of respiratory noises.

CONCLUSION
The NOEL for the dam was established as 100 mg/kg bw/day of test substance (equivalent to 69 mg/kg bw/day notified chemical), based on signs of respiratory distress and deaths at 300 and 1000 mg/kg bw/day.

Despite the slightly higher risk of irritation to the respiratory tract at doses of 300 mg/kg bw/day and above, it was concluded that 300 mg/kg bw/day of test substance (equivalent to 207 mg/kg bw/day notified chemical) was the NOAEL for the dam. Effects on body bodyweight gain and food consumption were also observed at 1000 mg/kg bw/day.

The NOEL for the foetus was established as 1000 mg/kg bw/day of test substance (691 mg/kg bw/day notified chemical), based on no adverse effects observed at the highest dose tested (1000 mg/kg bw/day).

TEST FACILITY Huntingdon Life Sciences Ltd (1999)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated sludge from sewage treatment works (Thorndon) handling predominantly domestic sewage
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biological oxygen demand
Remarks - Method	The notified chemical was tested at 3 mg/L, and the reference substance at 5 mg/L

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	44	5	55
28	45	11	68

Remarks - Results	Biodegradation of the test substance reached a plateau after 5 days. The test substance was not inhibitory to the microbial inoculum at the concentration tested, based on the results from a toxicity control containing test and reference substances.
CONCLUSION	Not readily biodegradable
TEST FACILITY	Huntingdon Life Sciences Ltd (2000e)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: Modified Sturm Test.
Inoculum	Activated sludge from sewage treatment works (Eye) handling predominantly domestic sewage
Exposure Period	29 days
Auxiliary Solvent	None
Analytical Monitoring	Evolution of carbon dioxide
Remarks – Method	Test and reference substances were tested at 10 mg/L carbon

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	10	1	11
8	64	11	81

Remarks - Results	Biodegradation of the test substance reached 89% after 29 days. The test substance was not inhibitory to the microbial inoculum at the concentration tested, based on the results from a toxicity control containing test and reference substances.
CONCLUSION	Readily biodegradable

TEST FACILITY Huntingdon Life Sciences Ltd (2003c)

C.1.3. Bioaccumulation

No bioaccumulation test was conducted. The notified chemical is not expected to bioaccumulate in fish as it is highly water soluble and biodegradable.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – flow-through.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – flow-through.

Species	Zebra fish (*Danio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	~ 150 mg $CaCO_3$/L
Analytical Monitoring	HPLC with UV detection (LOQ 0.25 mg/L)
Remarks – Method	Stock solutions were freshly prepared at 24 hour intervals

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*2 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	< LOQ	10	0	0	0	0	0
10	0.36-6.64	10	1	1	1	1	1
17.8	0.86-8.36	10	0	0	0	0	0
31.6	3.49	10	2	10	10	10	10
56.2	n/a	10	10	10	10	10	10
100	n/a	10	10	10	10	10	10

LC50	23.7 mg/L at 24 hours 23.7 mg/L at 48 hours 23.7 mg/L at 72 hours 23.7 mg/L at 96 hours
NOEC	10 mg/L at 96 hours
Remarks – Results	The mortality at 10 mg/L was not related to the test substance. Sublethal effects (fish at bottom of aquarium after 2 hours) were seen at 17.8 mg/L. Measured concentrations declined between renewals of the stock solution, but the magnitude of this decline was highly inconsistent as indicated by the data tabulated above. The results are expressed as nominal concentrations.

CONCLUSION The notified chemical is harmful to fish

TEST FACILITY CIT (2002)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static.

Species	*Daphnia magna*

Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	246 mg/L $CaCO_3$/L
Analytical Monitoring	HPLC with UV detection (LOQ 0.028 mg/L)
Remarks - Method	

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	< LOQ	20	0	0
0.43	0.30	20	0	0
0.94	0.75	20	0	0
2.07	1.66	20	0	0
4.55	4.00	20	0	0
10	8.98	20	17	18

LC50	6.76 mg/L at 24 hours 6.54 mg/L at 48 hours
NOEC	4.00 mg/L at 48 hours
Remarks - Results	Results are expressed as initial measured concentrations. The test substance could not be measured in any of the 48 hour samples. Preliminary stability analyses indicated that the test substance was lost from aqueous solution with the passage of time.

CONCLUSION The notified chemical is toxic to daphnids.

TEST FACILITY Huntingdon Life Sciences Ltd (2001g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species	*Selenastrum capriconutum*
Exposure Period	72 hours
Concentration Range	Nominal: 0.064 - 1.5 mg/L Actual: < LOQ – 1.25mg/L
Auxiliary Solvent	None
Water Hardness	Typical algal nutrient medium containing various nutrients including 18 mg/L calcium chloride dihydrate
Analytical Monitoring	HPLC (same method as for daphnid test)
Remarks - Method	

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
0.46	0.24	0.72	0.24

Remarks - Results	Analytical data indicated that the test substance was lost from the test medium during the test, except at the highest concentration where algal growth was almost completely inhibited. The results tabulated above are expressed as initial measured concentrations.

CONCLUSION The notified chemical is very toxic to green algae.

TEST FACILITY	Huntingdon Life Sciences Ltd (2001h)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge from sewage treatment works (Oakley) handling predominantly domestic sewage
Exposure Period	3 hours
Concentration Range	0, 30, 50, 100 and 300 mg/L
Remarks – Method	3,5-Dichlorophenol was used as reference substance
RESULTS	
IC50	98.5 mg/L (95% CI 86.6-112.9 mg/L)
NOEC	< 30 mg/L. IC20 and IC 80 values were 60 and 150 mg/L.
Remarks – Results	The sensitivity of the reference substance fell within the normal range.
CONCLUSION	The test substance is inhibitory to microbial respiration at the tested concentrations
TEST FACILITY	Huntingdon Life Sciences Ltd (2000f)

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